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ARCHITECTURE AND FRUITING OF APPLE TREE IN AGROFORESTRY – LINKING ARCHITECTURAL DEVELOPMENT, FLOWERING AND XYLEM FLOW.

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ARCHITECTURE AND FRUITING OF APPLE TREE IN AGROFORESTRY – LINKING ARCHITECTURAL DEVELOPMENT, FLOWERING AND XYLEM FLOW.

ABSTRACT

Agroforestry systems structured around fruit trees to produce fresh fruit is still under-developed in temperate zones. This study is based on the idea that the fruit tree can be integrated into multi-strata agroforestry systems where it would be grown with timber trees occupying the upper stratum and shrubs and/or herbaceous plants in the lower stratum. In addition to the production of fresh fruit, such systems would then combine different agro-ecosystem services over a longer period (timber production) or over shorter periods of time (possibly wood-energy production; regulation of pests and/or nitrogen enrichment of the soil). Beside the potential interest of plant biodiversity managed in this way, aimed at reducing dependence on pesticides, these systems would be potentially interesting in the Mediterranean area in order to limit the harmful effects of excessive summer radiation (temperature and light) on the fruit tree that cause photo-oxidative stress at the foliar level and fruit sunburns.

The study focuses on a major temperate fruit species at the national and world scales, the apple tree. The experimental plot is located at the Restinclières estate (UMR ABSys, Montpellier), where apple trees were planted in 2016 at different distances from 21-year-old walnut trees, thus establishing a competition gradient for light as well as for water and mineral elements. The general objective wass to acquire a detailed knowledge of the tree architectural development, its flowering and fruit-set, along these competition gradients. The work focuses on three actions: (i) defining an indicator to characterize each apple tree environment in this complex agrosystem, (ii) analyse at the tree scale the impact of agroforestry on architectural, morphological and phenological traits, and (iii) analysing the daily and annual sap flow with regard to environmental variables.

Our hypothesis is that the competition gradient induced by our agrosystem, especially for light, leads to phenological desynchronization in apple trees and variations in resource allocations between the vegetative and reproductive compartments of the tree and affect fruit set. This work has shown that considering light, hypothesized as the limiting factor, was more relevant to characterize each apple trees than usual distance-dependent crowding index. Using the light as a variable to analyse our architectural data, we have shown that apple trees did express shade avoidance traits affecting morphology (decreased taper, and increased slenderness and specific leaf area), architecture (fewer growing shoots and proportion of flower clusters) and phenology (reduced number of days at full bloom). Finally, we have shown that sap flow, water use and transpiration per unit of leaf area was affected by environmental variables (vapour pressure deficit and reference evapotranspiration). Shade did not change apple trees sap flow daily dynamics and reduced water and transpiration per unit of leaf area mainly because of a reduced Huber value (ratio of sap wood area on leaf area) in our experimental conditions. A shade related decrease in leaf area or in the number of ramifications was correlated to a decrease in transpiration per unit of leaf area during the summer. Our results suggest that while the architecture of apple trees is modified by a reduction in light intensity, it is not until a reduction of 65% that the capability to set fruit is impeded.

KEY WORDS

Apple tree, Agroforestry, Architecture, Sap flow, Flowering, Shade.

ARCHITECTURE ET MISE A FRUIT DU POMMIER EN SYSTEME AGROFORESTIER - COUPLAGE ENTRE DEVELOPPEMENT ARCHITECTURAL, FLORAISON, FRUCTIFICATION ET FLUX XYLEMIEN.

RESUME

Les systèmes agroforestiers structurés autour des arbres fruitiers pour la production de fruits frais sont encore peu développés dans les zones tempérées. Cette étude est basée sur l'idée que l'arbre fruitier peut être intégré dans des systèmes agroforestiers multi-strates où il serait cultivé avec des arbres à bois d'œuvre occupant la strate supérieure et des arbustes et/ou des plantes herbacées dans la strate inférieure. Outre la production de fruits frais, ces systèmes combineraient alors différents services agro-écosystémiques sur une période longue (production de bois d'œuvre) ou courte (éventuellement production de bois-énergie ; régulation des parasites et/ou enrichissement du sol en azote). Outre l'intérêt potentiel d'une biodiversité végétale ainsi gérée, visant à réduire la dépendance aux pesticides, ces systèmes seraient potentiellement intéressants dans la zone méditerranéenne afin de limiter les effets néfastes d'un rayonnement estival excessif (température et lumière) sur l'arbre fruitier qui entraine des stress photo-oxydatifs au niveau foliaire et des brulures sur fruit.

L'étude se concentre sur une espèce fruitière tempérée majeure aux niveaux national et mondial, le pommier. La parcelle expérimentale est située sur le domaine des Restinclières (UMR SYSTEM, Montpellier), où des pommiers ont été plantés en 2016 à différentes distances des noyers plantés en 1998, établissant ainsi un gradient de compétition pour la lumière, l'eau et les éléments minéraux. L'objectif général est d'acquérir une connaissance détaillée de l'établissement architectural de l'arbre, de sa floraison et de sa nouaison, le long de ces gradients de compétition. Le travail se concentre sur trois actions : (i) définir un indicateur pour caractériser l'environnement de chaque pommier dans cet agrosystème complexe, (ii) analyser à l'échelle de l'arbre l'impact de l'agroforesterie sur les caractéristiques morphologiques, phénologiques et architecturales, et (iii) analyser la dynamique de densité de flux de sève et le cumul annuel en fonction des variables environnementales et en relation avec les caractéristiques architecturales susmentionnées.

Notre hypothèse est que le gradient de compétition induit par notre agrosystème, en particulier pour la lumière, entraîne une désynchronisation phénologique chez les pommiers et des variations dans l'allocation des ressources entre les compartiments végétatif et reproductif de l'arbre affectant le potentiel de production de fruits. Ce travail a montré que la prise en compte de la quantité lumière, considérée comme le facteur limitant, était plus pertinent pour caractériser l'environnement de chaque pommier qu'un indice de voisinage. En utilisant la lumière comme variable pour analyser nos données architecturales, nous avons montré que les pommiers exprimaient effectivement des traits d'adaptation à l'ombre affectant la morphologie (diminution de la conicité et augmentation de l'élancement et de la surface foliaire spécifique), l'architecture (moins de pousses en croissance et d'inflorescences) et la phénologie (réduction de la période de pleine floraison). Enfin, nous avons montré que la densité de flux de sève n'était pas affectée par des variables environnementales (déficit de pression de vapeur et l'évapotranspiration de référence) contrairement à la transpiration par unité de surface foliaire. L'ombre n'a pas modifié la dynamique quotidienne de densité de flux de sève des pommiers et a réduit l'eau et la transpiration par unité de surface foliaire, principalement en raison d'une adaptation morphologique et architecturale dans nos conditions expérimentales. Une réduction de la surface foliaire ou simplification de l'architecture du pommier (réduction du nombre de ramifications) a diminué la transpiration par unité de surface foliaire pendant l'été. Nos résultats suggèrent que si l'architecture des pommiers est modifiée par une réduction de l'intensité lumineuse, ce n'est qu'à partir d'une réduction de 65% que la capacité à produire des fruits est entravée.

MOTS CLEFS

Pommier, Agroforesterie, Architecture, Ombre, Flux de sève, Floraison.

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RESUME ETENDU

APPROCHE

L'agriculture doit nourrir une population mondiale croissante tout en préservant l'environnement et la santé humaine. Dans un avenir proche, nos systèmes agricoles devront également s'adapter au réchauffement climatique qui va entrainer davantage de phénomènes météorologiques extrêmes tels que des sécheresses et des inondations, en plus d'une augmentation des maladies et des ravageurs. Après la seconde guerre mondiale, l'intensification de l'agriculture a permis d'augmenter le rendement des cultures grâce à la sélection génétique et une dépendance croissante vis-à-vis des intrants (nutriments, pesticides, irrigation).

Les vergers de pommiers ne font pas exception à la règle et leur intensification, si elle a permis d'augmenter considérablement leur productivité, a reposé sur une utilisation croissante de produits chimiques. Cette dépendance à l'égard des intrants chimiques et leurs effets néfastes sur l'environnement et la santé humaine sont considérés comme un obstacle à l'intensification durable de l'agriculture en général. La pomme reste l'une des cultures fruitières les plus traitées, qui nécessite entre 10 et 24 applications pendant une saison. En outre, une forte réduction du nombre de cultivars a accompagné cette intensification, augmentant le risque de réduire la capacité génétique à résister ou à tolérer les ravageurs existants et nouveaux, et à s'adapter au changement climatique. Pour réduire cette dépendance aux intrants, les vergers monoclonaux ne sont guère appropriés. Par conséquent, la gamme des cultivars de pommes existants et la conception du système de vergers de pommiers doivent être reconsidérés.

Les systèmes agroforestiers, en tant que combinaison intentionnelle sur une même parcelle d'arbres et/ou d'arbustes avec des cultures annuelles ou du bétail, constituent une bonne opportunité de re-conception des agrosystèmes et sont reconnus comme une pratique agricole durable depuis un demi-siècle. Les bénéfices reconnus de l'agroforesterie comprennent la réduction de la lixiviation des nutriments et des pesticides, l'amélioration de la qualité des sols, le contrôle de l'érosion, la séquestration du carbone et l'atténuation des risques liés aux événements climatiques extrêmes. Par ailleurs, si elle intègre un choix réfléchi de plantes associées (répulsives des bio-agresseurs ou attractives d'arthropodes prédateurs) l'agroforesterie peut également permettre l'augmentation du biocontrôle,

Les systèmes agroforestiers à base de pommiers pourraient être une solution intéressante pour réduire l'utilisation des pesticides, car les vergers monoclonaux à haute densité actuels sont généralement très sensibles aux parasites et aux maladies et, par conséquent, dépendants des pesticides. Outre les intérêts susmentionnés, un système agroforestier à base de pommiers pourrait également être intéressant dans la région méditerranéenne pour le pommier en limitant les effets néfastes du rayonnement estival excessif récurrent (lumière et température), qui est responsable de stress oxydatif au niveau foliaire, et de brulures sur fruits provoquant des pertes annuelles parfois importantes dans les vergers. En effet, dans ces régions régulièrement exposées à un fort rayonnement solaire et à un climat sec, même si la compétition pour la lumière et les nutriments peut affecter la croissance des pommiers, les principaux bénéfices attendus sont l'atténuation des stress microclimatiques. Toutefois, la complexité inhérente aux systèmes agroforestiers est le principal obstacle à la réalisation de leurs avantages potentiels. Pour optimiser un système agroforestier, outre la sélection d'espèces sans effets allélopathiques ou à faible compétition interspécifique, il est nécessaire de les étudier de manière approfondie pour en tirer des règles d'assemblage temporelles et spatiales.

Si les systèmes agroforestiers basés sur les pommiers doivent être adoptés par les agriculteurs en climat tempéré, et bien qu'une culture fortement rémunératrice puisse faciliter son adoption, de nombreux défis restent à relever et notamment la capacité à produire suffisamment de fruits en quantité et en qualité. À notre connaissance, il n’y a encore que très peu d’études sur les effets de l’ombrage sur l’architecture (expression de la ramification) et la morphologie des pousses (longueur, forme) du pommier, les interactions avec la floraison et la fructification, et l'utilisation de l'eau par l’arbre. Mon étude a été réalisée sur de jeunes pommiers, durant leurs troisièmes et quatrièmes années de croissance, poussant à différentes distances de noyers. Cette phase de la vie de l’arbre fruitier est en effet cruciale car elle préfigure le volume de l’arbre adulte et son potentiel de fructification.

Ma recherche a été développée autour de 3 objectifs. Le premier objectif était de caractériser l'environnement des pommiers qui était planté selon trois modalités, témoin agricole (AC), agroforesterie inter-rang (AFIR) et agroforesterie rang (AFR). Dans la présente étude, un modèle de l'encombrement de la canopée dépendant de la distance a tout d’abord été élaboré à partir de la littérature (Neighbourhood Crowding Index, NCI) pour caractériser l'encombrement de chaque pommier sans tenir compte d'un facteur limitant spécifique. Cependant, dans les couverts denses en général, et dans l'agroforesterie plus spécifiquement, il a été démontré que la lumière était un facteur critique pour les cultures des strates inférieures. J’ai donc, dans un deuxième temps, quantifié le rayonnement photosynthétiquement actif (PAR) atteignant le pommier, considérant que c'était une condition préalable pour comprendre les effets du contexte agroforestiers sur la physiologie et l'architecture du pommier. J'ai utilisé deux méthodes pour estimer les régimes lumineux du pommier sous les couverts de la couche supérieure en utilisant la photographie hémisphérique ou des scanners terrestres. La première partie de ce travail commence par (i) comparer les méthodologies pour estimer la quantité de PAR reçue par les pommiers cibles, et (ii) estimer lesquels des PAR, calculés à partir des photographies hémisphériques ou des scanners terrestres, ou du NCI sont les plus à même d'expliquer la variabilité des caractères végétatifs et reproductifs mesurés sur ces pommiers.

Le deuxième objectif était de déterminer (i) quels sont les traits de la croissance végétative qui sont affectés par un gradient de réduction de la lumière atteignant le pommier, et (ii) plus spécifiquement, si et comment l'ombre a un impact sur sa floraison et de fructification. Notre méthodologie comprenait la quantification des traits morphologiques et architecturaux, à l'échelle de la pousse et de l'arbre entier respectivement, ainsi que les traits phénologiques des inflorescences. Comme les effets de l'ombre sont complexes et seulement partiellement documentés sur les plantes pérennes, les changements attendus dans la morphologie du pommier (augmentation de la surface spécifique des feuilles, allongement des entre-noeuds et des axes), l'architecture (diminution du nombre de ramifications et des inflorescences) et la phénologie (modification de la phénologie des inflorescences et du taux de croissance relatif des pousses) seront spécifiquement étudiés ici et discutés à la lumière des connaissances sur les espèces annuelles adoptant des stratégies d'évitement et de tolérance.

Le troisième objectif de cette étude était (i) de quantifier la densité de flux de sève (J_s), l'utilisation de l'eau (WU) et la transpiration par unité de surface foliaire (E_l) des pommiers, (ii) de déterminer comment l'environnement lumineux influençait J_s et E_l, et (iii) d'étudier les relations entre l'architecture des pommiers à l'échelle de l'arbre, et J_s ou E_l. Les étés en climat méditerranéen sont caractérisés par un rayonnement et une température élevés qui conduisent les pommiers à fermer leurs stomates et donc à réduire le fonctionnement photosynthètique. Nous avons émis l'hypothèse que J_s et E_l pour les pommiers plantés à proximité des noyers seraient peu impacté pendant la saison de croissance. Bénéficiant d'un microclimat favorable et d'un rayonnement moins excessif, nous nous attendons à ce que les pommiers à l'ombre maintiennent leurs stomates ouverts plus longtemps que les pommiers en pleine lumière. Par conséquent, considérant une journée d'été où le déficit de pression de vapeur (D) est élevé J_s devrait être plus élevés pour les pommiers en agroforesterie que pour les pommiers en plein soleil. Enfin, comme les différents degrés d'ombrage ont un impact sur l'architecture des pommiers, nous avons émis l'hypothèse que ces différences d'architecture pourraient être liées à l'E_I des pommiers.

RESULTATS

(1) Une évaluation critique du Neighbourhood Crowding Index : application au pommier cultivé dans un système agroforestier multistrates.

Nous avons commencé par comparer deux méthodologies pour estimer, sur une même période, le PAR calculé à partir de photos hémisphériques (PAR_{HP}) ou à partir de nuages de points issus d'un scanner terrestre (PAR_{TLS}). Afin de comparer les deux méthodologies pour estimer le PAR atteignant chacun des 45 pommiers, une corrélation entre PAR_{HP} et PAR_{TLS} a été réalisée. Le PAR_{HP} explique 61% de la variabilité de PAR_{TLS} et il n'y a pas de biais entre les deux méthodologies ($r^2 = 0,61$, nRMSE = 0,47, nMBE = -0,02). En comparant les résultats de chaque traitement, les pentes entre l'agroforesterie rang (AFR) et l'agroforesterie inter-rang (AFIR) ne sont pas significativement différentes (t = 1,6, df = 2, P = 0,2), mais les pentes entre le témoin agricole (AC) et AFIR (t = 18,9, df = 2, P = 1,3e-05), et AC et AFR (t = 33,7, df = 2, P = 6,4e-09) étaient significativement différentes.

Pour déterminer quel indicateur environnemental, NCI, PAR_{TLS}, PAR_{HP}, était le meilleur indicateur pour expliquer l'impact de l'environnement des pommiers sur leur croissance et leur développement, des corrélations avec la surface foliaire et le nombre d'inflorescences en 2018 et 2019 ont été effectuées. PAR_{TLS} est toujours le meilleur indicateur pour expliquer la variabilité des caractères considérés, sauf pour le nombre d'inflorescence en 2018 n'expliquant que 45 % de la variabilité. PAR_{HP} est toujours meilleur que le NCI pour expliquer la variabilité des caractères considérés. Dans le cas de la surface foliaire, PAR_{TLS} explique 63% et 64% de la variabilité en 2018 et 2019, respectivement, alors que PAR_{HP} explique 47% et 44% et NCI 36% et 38%. En outre, PAR_{TLS} explique 57% et 86% de la variabilité du nombre d'inflorescence en 2018 et 2019, tandis que PAR_{HP} explique 48% de la variabilité pour les deux années et NCI 47% et 46%.

(2) Adaptation du pommier à l'ombre en système agroforestier – une approche architecturale.

La première étude nous a permis de grouper les différents pommiers en fonction de la quantité de lumière reçu. La diminution de la quantité de lumière reçue par les pommiers n'a pas affecté la hauteur du tronc en 2019, mais il y a eu une diminution significative de sa section transversale du tronc. Cela a entraîné une différence dans l'élancement et la conicité. L'élancement était significativement plus élevé en agroforesterie qu'en pleine lumière. La conicité, en revanche, était significativement plus élevée en pleine lumière qu'en agroforesterie. Il n'y a pas de différences statistiquement significatives dans la longueur des entre-nœuds des pousses. La surface foliaire spécifique était affectée par l'intensité lumineuse, les feuilles en plein soleil ayant une surface foliaire spécifique significativement plus faible que les feuilles sur les arbres en lumière modérée et faible.

Bien qu'il y ait eu une forte augmentation à la fois du nombre et de la proportion d’inflorescences entre 2018 et 2019, l'effet de l’ombre a été similaire sur les deux années pour tous les pommiers entrainant une diminution significative de l'initiation florale. En 2018, il y a eu une différence significative dans le nombre et la proportion des inflorescences entre les pommiers en pleine lumière et en lumière modérée d’une part, et les arbres en faible lumière d’autres part. En 2019, il y a eu une différence significative dans le nombre d’inflorescence, entre les pommiers en pleine lumière et les arbres en lumière modérée d'une part, et les arbres en faible lumière d'autre part. Cependant, les pommiers en pleine lumière avaient une proportion significativement plus élevée d’inflorescences que les pommiers en faible lumière. Les pommiers en lumière modérée n'étaient pas significativement différents des deux autres groupes. Nous avons également observé que le nombre d’inflorescence de l’année N était positivement corrélé avec la surface foliaire de l'année N-1 pour les deux couples d’années étudiés, avec des différences, toutefois, en fonction du contexte lumineux. En pleine lumière, la corrélation était très significative pour les deux années. 50 % de la variabilité du nombre d’inflorescence en 2019 s'expliquait par la surface foliaire de l'arbre en 2018 et passait à 71 % en 2020 lorsqu'elle était corrélée à la surface foliaire de l'arbre en 2019. Dans un contexte de lumière modérée, la corrélation était également significative pour les deux années, mais la surface foliaire du pommier de l'année précédente n'expliquait que 37 % de la variabilité du nombre d’inflorescence en 2019 et 31 % en 2020. Dans une lumière faible, la corrélation entre la surface foliaire des pommiers de l'année précédente et le nombre d’inflorescence de l'année en cours n'était pas significative pour les deux années.

À une occasion, en 2018, on a constaté une différence significative dans la phénologie des inflorescences entre la pleine lumière et la lumière modérée. La phénologie des inflorescences était significativement plus avancée en lumière modérée 37 jours après le débourrement que celle en pleine lumière. La chute des pétales s'est produite plus rapidement en lumière modérée. Pour les bourgeons floraux en faible lumière, il y avait une différence significative par rapport à la pleine lumière 32 jours après le débourrement. La chute des pétales s'est produite de manière précoce et plus rapidement pour les inflorescences en faible lumière par rapport à la pleine lumière. Il n'y a pas eu de différences statistiquement significatives dans les stades phénologiques des inflorescences en lumière modérée et faible lumière. En 2019, il y avait une différence significative dans la phénologie des inflorescences entre la pleine lumière et la lumière modérée 35, 40 et 42 jours après le débourrement. Les stades phénologiques concernés correspondaient à la chute des pétales et à la nouaison. Une fois de plus, la chute des pétales s'est produite plus rapidement en lumière modérée. Pour les bourgeons floraux en lumière faible, on a constaté une différence significative dans la phénologie des inflorescences 37, 40 et 42 jours après le débourrement. Il s'agissait des mêmes stades phénologiques. La chute des pétales s'est produite plus rapidement mais pas aussi précocement qu'en 2018.

(3) L'agroforesterie peut-elle améliorer la transpiration des pommiers ? - Un essai combinant variables environnementales et flux de sève.

Nos résultats montrent que la densité du flux de sève (J_s) ne semblait pas suivre la pression de vapeur saturante (D) et l'évapotranspiration de référence (ET_0) et n'était pas significativement différente entre les différents degré d'ombrage en 2018 et 2019. En outre, il n'y a pas eu de différences nettes entre les pommiers plantés dans les différentes conditions lumineuses, sauf pendant la deuxième moitié de septembre où les J_s cumulés des pommiers en pleine lumière étaient plus élevés que ceux des pommiers en conditions ombragées. En examinant les J_s cumulés en 2019, les pommiers plantés en condition ombragée avaient une densité de flux de sève plus élevée que les pommiers plantés en pleine lumière en mai, mais cette différence a disparu en septembre lorsque les J_s étaient plus élevés pour les pommiers en pleine lumière.

La transpiration (J_s x SWA (sap wood area)) était significativement différente entre les pommiers plantés dans les différentes conditions lumineuses en 2018 et 2019. Les pommiers plantés en pleine lumière avaient une transpiration plus élevée que les arbres en lumière modérée qui, à leur tour, avaient une transpiration plus élevée que les pommiers en faible lumière. La différence significative dans l'utilisation de l'eau était donc principalement la conséquence d'une différence significative de SWA entre les arbres soumis à des contextes lumineux différents. En effet, SWA était significativement différent entre tous les traitements à la lumière en 2018, les pommiers en pleine lumière ayant une SWA plus élevée que les pommiers en lumière modérée qui, à leur tour, avaient une SWA plus élevée que les pommiers en faible lumière. En 2019, les pommiers en pleine lumière avaient encore une SWA significativement plus élevée que les pommiers en faible lumière et les pommiers en lumière modérée n'étaient pas significativement différents. En 2018, les différences dans l'utilisation de l'eau étaient vraiment marquées pendant les mois de juillet et septembre. En 2019, la différence dans l'utilisation de l'eau était importante depuis le mois de mai jusqu'à la fin de la saison de croissance.

Les pommiers en lumière modérée et faible étaient moins sensibles à une augmentation de D que les pommiers en pleine lumière, ce qui suggère que la proximité des noyers induite par l'ombre a limité la valeur maximale de J_s le matin. 78 % et 88 % de la variabilité de la transpiration (WU) pendant l'été s'expliquait par la surface foliaire en 2018 et 2019 respectivement. En effectuant cette corrélation pour chaque traitement, la relation était encore très significative pour les pommiers en pleine lumière et en lumière modérée, mais plus pour les pommiers en faible lumière. La surface foliaire expliquait 95 % et 69 % de la WU pendant l'été pour les pommiers en pleine lumière en 2018 et 2019 respectivement, et 53 % et 66 % pour les pommiers en lumière modérée. Les pommiers en pleine lumière avaient une surface foliaire plus élevée que les pommiers en lumière modérée et, par conséquent, une WU plus élevée pendant l'été. Cependant, bien que la surface foliaire et le nombre de ramifications expliquent respectivement 40 % et 50 % de la variabilité de l'E_l cumulé en 2019. Nous avons ensuite corrélé E_l à la surface foliaire ou au nombre de ramifications pendant l'été 2019, lorsque le stress thermique et les différences étaient les plus importants. Une relation logarithmique était la plus appropriée pour ces corrélations et expliquait respectivement 40 % et 50 % de la variabilité de l'E_l cumulé.

DISCUSSION

Pour optimiser les systèmes agroforestiers (AFS) en général, il est essentiel de comprendre les interactions entre les plantes afin de maximiser les interactions positives et de minimiser les interactions négatives. Notre étude a montré que lorsqu'on travaille dans des systèmes agroforestiers complexes, l'utilisation d'un indicateur environnemental continu qui aide à caractériser les interactions pour chaque arbre peut compléter l'analyse en utilisant uniquement les traitements comme facteur indépendant en raison de l'hétérogénéité au sein d'un traitement. Les modèles d'encombrement dépendant de la distance, comme le Neighbourhood Crowding Index (NCI), se sont avérés pertinents pour modéliser les caractéristiques architecturales des arbres, mais moins que la prise en compte du facteur limitant (c'est-à-dire la lumière dans cette étude) et sa quantification. Le NCI considère qu'il n'y a pas de facteur limitant spécifique, mais qu'un certain nombre de ressources limitent simultanément la croissance des pommiers. La lumière étant supposée être le facteur limitant dans cette étude, nous avons utilisé deux méthodologies pour évaluer le rayonnement photosynthétiquement actif (PAR). La première a estimé le PAR reçu pendant la saison de croissance en utilisant des photographies hémisphériques (PAR_{HP}) et la seconde le PAR reçu pendant la pleine foliation, c'est-à-dire lorsque l'ombre était maximale, en utilisant un scanner terrestre (PAR_{TLS}).

Nous avons montré que les méthodologies utilisées pour quantifier le facteur limitant, la lumière, doivent être considérées au préalable et choisies de manière adéquate en fonction des objectifs et des ressources de l'étude. L'utilisation du LIDAR terrestre qui permet de calculer à la fois le volume de la canopée des arbres et le PAR interceptée est plus précise et plus exacte que l'utilisation de photographies hémisphériques, mais son coût est plus élevé et son analyse plus longue. Dans la présente étude, le système agroforestier intégrant du pommier est encore jeune et l'équilibre entre les interactions en surface et souterraines pourrait encore se modifier, surtout si l'on considère que l'irrigation au goutte-à-goutte favorise la présence des systèmes racinaires dans la même zone que les arbres voisins. Dans ce cas, un indicateur qui englobe la lumière, le volume de la canopée et la concurrence souterraine pourrait être plus intéressant et plus robuste à l'avenir.

Dans un système agroforestier basé sur les arbres fruitiers, l'objectif est de produire des fruits commercialisables, nous avons étudié deux étapes essentielles : l'initiation florale et la nouaison des fruits. Cette dernière est le premier obstacle qui déterminera le rendement au cours d'une saison de croissance. Il a été signalé que la floraison est accélérée ou retardée par l'ombre selon l'expérience et le matériel végétal. Notre étude est en partie en désaccord avec ces résultats. Nous avons montré que si la date de débourrement n'était pas liée à l'intensité lumineuse, c'est peut-être parce que le noyer étant une espèce à débourrement tardif, les différences de conditions lumineuses (dues au tronc et aux branches) sont moins marquées à cette date. La phénologie a été modifiée autour de la pleine floraison. En 2018 et 2019, les inflorescences à l'ombre ont perdu leurs pétales prématurément par rapport aux inflorescences en pleine lumière. Cela pourrait conduire à une moindre attractivité pour les pollinisateurs et donc à une fenêtre de pollinisation plus courte, et pourrait potentiellement affecter négativement la nouaison. Cependant, dans notre étude, l'intensité lumineuse a réduit de manière significative le taux de nouaison en pleine lumière par rapport à l'ombre, ce qui suggère que la chute des pétales a eu un impact limité sur le taux de nouaison. L'ombre a même favorisé la nouaison du pommier, mais ce résultat pourrait être la conséquence d'une charge de fruits plus faible à l'échelle de l'arbre.

Une diminution de l'intensité lumineuse, qu'elle soit naturelle ou artificielle, a un impact négatif sur l'initiation florale. L'initiation florale est un phénomène complexe, mais on sait qu'elle est corrélée à la surface foliaire de l'année précédente. Dans notre étude, une réduction d'environ 30 % de l'intensité lumineuse a réduit de manière significative la surface foliaire pour les deux années, 2018 et 2019, et le nombre d'inflorescence seulement en 2019. Bien que notre étude n'ait été réalisée que sur deux ans, cela suggère que les différences entre les pommiers dans différentes conditions de lumière seront plus marquées avec le vieillissement. Le manque de lumière pourrait également prolonger la période durant laquelle le pommier établit son architecture avant qu'il n'entre en pleine production de fruit, ce qui, à son tour, influencerait sa régularité de floraison. Cependant, la relation entre la surface foliaire de l'année précédente et le nombre d'inflorescences de l'année en cours n'a pas été modifiée entre la pleine lumière et la lumière modérée. Ces deux variables ont été positivement corrélées en 2019 et 2020. Le manque de photosynthétat dû à une ombre modérée ralentit la croissance et le développement de l'arbre mais ne semble pas affecter sa capacité de fructification par rapport au développement végétatif. D'autres années d'étude seront nécessaires avant de conclure, mais ces résultats sont prometteurs pour l'avenir des systèmes agroforestiers basés sur les pommiers.

Certaines études ont souligné l'intérêt d'utiliser des filets d'ombrage ou de protection contre la grêle pendant les périodes critiques pour atténuer les luminosités et chaleurs extrêmes. Notre étude a révélé que la densité quotidienne moyenne du flux de sève (c'est-à-dire le flux de sève par unité de surface de xylème, J_s) n'était pas affectée par les conditions environnementales, mais que l'utilisation de l'eau et la transpiration par unité de surface foliaire l'étaient. L'utilisation de l'eau (WU) ainsi que la transpiration par unité de surface foliaire (E_l) était négativement influencée par l'ombre. Cependant, la dynamique du flux de sève, J_s (dynamique quotidienne mesurée toutes les heures) a été affectée par l'environnement, en particulier pendant l'été. Les pommiers en lumière modérée et faible étaient moins sensibles à une augmentation de la pression de vapeur maximale journalière (D_{max}) que les pommiers en pleine lumière. Ces résultats suggèrent que l'ombre ou la proximité des noyers ont limité la valeur maximale de J_s le matin.

Sachant que les conditions lumineuses n'ont eu aucun effet sur le cumul de J_s pendant la saison de croissance, les différences d'utilisation de l'eau étaient dues à des différences de morphologie des pousses et d'architecture des pommiers. La biomasse de l'appareil végétatif aérien a été réduite à l'ombre pendant les deux années, ce qui est probablement la conséquence d'une réduction du taux de photosynthèse, la quantité de lumière n'étant pas suffisante pour saturer les photorécepteurs foliaires. Le fait que des différences significatives dans la biomasse aérienne sèche aient disparu entre 2018 et 2019 pourrait indiquer que ces différences pourraient diminuer avec l'ontogenèse des pommiers et leur architecture qui se complexifie. Toutefois, nous n'avons pas intégré ici la biomasse des fruits alors qu'ils constituent un puits important de glucides, ce qui a entraîné une sous-estimation de la biomasse aérienne pour tous les pommiers. Or, en 2019, 5 fruits par cm² de section transversale du tronc ont été laissés lorsque cela était possible et les pommiers en pleine lumière ont presque toujours atteint l'objectif alors que les pommiers en lumière modérée étaient très hétérogènes comme les pommiers en faible lumière. Nous pouvons en conclure que, comme ils portent davantage de fruits, la biomasse aérienne totale des pommiers en pleine lumière a été systématiquement sous-estimée par rapport aux pommiers en lumière modérée et faible.

E_l a été influencé par l'ombre et les variables environnementales. Les pommiers en pleine lumière étaient plus sensibles aux variables environnementales et les différences avec les pommiers dans d'autres environnements lumineux augmentaient avec des valeurs croissantes de D_{max} et d'ET_0. Cela suggère que, contrairement à ce qui a été notre hypothèse de départ, l'ombrage n'a pas augmenté E_l pendant les jours où la demande d'évaporation était élevée, mais qu'il l'a même entravée davantage. Bien qu'il puisse y avoir plusieurs raisons à ce comportement, nous avons observé que l'E_l cumulé pendant l'été 2019 était bien corrélé à la surface foliaire et au nombre de ramifications des pommiers. Nous faisons donc l'hypothèse que plus la surface foliaire augmente et plus l'architecture est complexe, plus les pommiers sont efficaces pour transpirer l'eau par unité de surface foliaire.

Ce travail a tenté de mettre en évidence l'intérêt potentiel des AFS à base de pommier dans le bassin méditerranéen en étudiant les effets, positifs et négatifs, d'une concurrence croissante entre arbres dominant le pommier et le pommier lui-même, son architecture, sa morphologie et son utilisation de l'eau. Nous avons montré que dans des conditions de faible luminosité, les interactions négatives sont trop fortes et limitent donc l'initiation florale et la capacité des pommiers à porter suffisamment de fruits. Cependant, une réduction modérée de la quantité de lumière (35% ici) a permis d'atteindre notre objectif de 5 fruits/cm² de section transversale du tronc dans certains cas, ce qui est un résultat prometteur. Dans notre étude, il est encore important de noter qu'aucun effet positif induit par la présence de noyers n'a été confirmé sur l'architecture, la morphologie ou l'utilisation de l'eau des pommiers. Pour évaluer précisément le potentiel de ces agrosystèmes, d'autres aspects doivent être évalués comme la régulation des bio-agresseurs, la biodiversité générée, le lessivage des nutriments, le carbone du sol, et comparés aux vergers de pommiers conventionnels en agriculture biologique ou en production fruitière intégrée et aux différentes conditions pédoclimatiques.

Cette thèse a permis d'acquérir des connaissances de base pour comprendre l'effet des interactions entre les plantes dans un système agroforestier à base de pommier. Nous avons montré que la lumière est un facteur environnemental important à considérer pour l'optimisation de cet agrosystème. Les pommiers étaient encore jeunes et n'étaient pas en pleine phase de reproduction, ce qui nous a permis d'analyser l'établissement architectural et fonctionnel du pommier qui est important puisque la forme et le volume général de l'arbre sont établis au cours de ces toutes premières années. Il y a encore beaucoup à apprendre de cette expérience, la régularité de production étant une problématique bien connue des arboriculteurs. Je pense qu'il est également important de mentionner que nos résultats sont très dépendants de nos conditions pédoclimatiques et ne peuvent donc pas être généralisés comme pour les expérimentations systèmes. Habituellement, le but de ces expérimentations est d'évaluer la capacité d'un système de culture à satisfaire des objectifs donnés tandis que les expériences factorielles servent à étudier et à comprendre l'effet d'un ou de quelques facteurs et de leurs interactions pris isolément, toutes choses étant égales par ailleurs, sur une ou plusieurs variables dépendantes. Alors que la première approche vise à évaluer un système global par rapport à des objectifs définis, la seconde peut contribuer à améliorer les agrosystèmes en améliorant un aspect technique ou une règle de décision. Dans ce travail, nous avons étudié comment un gradient de compétitions croissants impactaient la croissance et le développement des pommiers en considérant la lumière comme le facteur limitant et le facteur explicatif. Cela implique que nous avons travaillé dans le cadre d'une expérience "factorielle" pour établir une règle de décision afin de faciliter le prototypage de futur agrosystème identique. Bien que nos résultats nous permettent d’ores et déjà d'établir des lignes directrices de base, comme par exemple des distances minimales à respecter entre pommier et noyer, il faut garder à l'esprit que ces résultats sont très dépendants de notre parcelle expérimentale et qu'il faudrait un réseau de parcelles dans différentes conditions pédoclimatiques si nous voulions généraliser nos résultats.

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- **Pitchers B**, Do FC, Pradal C, Dufour L, Lauri PÉ. 2021. Apple tree adaptation to shade in agroforestry - An architectural approach. *American Journal of Botany* (in press).
- **Pitchers B**, Do FC, Lauri PÉ. 2021. A critical assessment of Neighourhood Crowding Index: application study in an agroforestry system of timber and fruit trees. *Applications in Plant Sciences* (in review).

COMMUNICATIONS

- **Pitchers B**, Dufour L, Lauri PÉ. 2020. Growing AgroForestry systems with Apple in Montpellier-Mediterranean - Preliminary results on the influence of adult walnut trees on growth and branching of two-year-old apple trees. *Acta Horticulturae* 1281: 323-330.
- **Pitchers B**, Do F, Lauri PÉ. 2019. A neighbourhood analysis to characterise competition in a multi-strata agroforestry system of timber and fruit tree. *In Dupraz, C., Gosme, M., Lawson, G. (Editors). 2019. Book of Abstracts, 4th World Congress on Agroforestry. Agroforestry: strengthening links between science, society and policy. Montpellier, France, 20-23 May 2019: CIRAD, INRA, World Agroforestry. 933 pages. P840.*
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CONTENTS

GLOSSARY

AC: Agricultural control

AFIR: Agroforestry inter row

AFR: Agroforestry row

AFS: Agroforestry system

AT-AFS: Apple tree agroforestry system

D: Vapour pressure deficit

E_l: Transpiration per unit of leaf area

FT-AFS: Fruit tree agroforestry system

J_s: Sap flow density

LA: Leaf area

NCI: Neighbourhood crowding index

PAR: Photosynthetically active radiation

RGR: Relative growth rate

SAS: Shade avoidance / adapatation syndrom

SLA: Specific leaf area

SMA: Standardized major axis

TCSA: Trunk cross section area

TLS: Terrestrial laser scanner

WU: Water use

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CHAPTER 1: *INTRODUCTION*

1. New stakes for agriculture

1.1. Intensification

Agriculture faces the task of feeding a growing world population while simultaneously having to avoid harmful effects on the environment and human health (Foley *et al.*, 2011). In the near future, our agricultural systems will also have to adapt to a changing climate that is expected to include more extreme weather events such as droughts and floods, in addition to an increase in disease and pest epidemics (Foley *et al.*, 2011). After the second world war the intensification of agriculture increased crop yield through breeding and an increasing dependence on inputs (i.e. nutrients, pesticides, water).

Apple orchards are no exception and their intensification, while it allowed to greatly increase their productivity, relied on an increasing use of chemicals (Lauri *et al.*, 2020). This reliance on chemicals inputs and their adverse effect on the environment and human health is considered as an obstacle to the sustainable intensification of agriculture in general (Lu *et al.*, 2015). Apple remains one of the most treated fruit crops that requires between 10 and 24 applications (Granatstein and Peck, 2017). Moreover, a strong reduction in the number of cultivars accompanied this intensification increasing the risk of narrowing the genetic ability to resist to or tolerate existing and new pests, and to adapt to climate change. To reduce this dependency to inputs monoclonal orchards are hardly appropriate (Simon *et al.*, 2011). Therefore, the range of existing commercial apple cultivars and the design of the apple orchard system need to be reconsidered (Lauri and Simon, 2019).

1.2. New paradigms for apple farming systems

The concept of ‘sustainability’ which was defined as “the ability to make development sustainable to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs” (Simon, 1987) has been the corner stone for several initiatives in the past decades in agriculture. Three apple farming systems have been developed in past decades towards these objectives: (i) Integrated production (IP), (ii) Organic farming (OF) and (iii) agroecology.

Applied to fruit, integrated fruit production (IFP) is defined as “the economical production of high quality fruit, giving priority to ecologically safer methods, minimizing the undesirable side effects and use of agrochemicals, to enhance the safeguards to the environment and human health” (Cross, 2002). IFP mainly aims at reducing the number of application of chemical towards pests by substituting them with biorational pesticides that express selectivity to specific developmental stages (e.g., biopesticides and insect growth regulators), semiochemicals (e.g., sex pheromones) and biological control (using natural enemies of pests) (Damos *et al.*, 2015). IFP is now considered as the standard conventional apple orchard in Europe where IPM is compulsory since 2009 (Damos *et al.*, 2015). Organic farming (OF) is defined as a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than on the use of inputs with adverse effects. In Europe, OF obeys to strict specifications that define precise farming and processing techniques (Migliorini and Wezel, 2017). A main difference between IFP and OF is that OF aims to manage the orchard system as a whole prohibiting synthetic inputs and their derivatives. However, OF may also raise environmental issues such as those related to intensive use of copper in humid climates or soil compaction due to more machinery traffic. Although the area of apple production in OF is increasing (Granatstein *et al.*, 2016), OF is still currently less developed than IFP in Europe (Evans, 2017). Eventually, agroecology aims at designing agrosystems which relies on ecosystem services. It combines knowledge of agronomy and ecology and has a defined set of principles for the ecological management of agrosystems. Agroecology meets an increasing interest in apple production since it opens routes to design novel apple-based systems optimizing interactions between the apple tree and the other plants of the system (Simon *et al.*, 2017).

Lovell *et al.* (2018) proposed the concept of “Multifunctional Woody polyculture” to name the association of different fruit tree species (and shrubs) and/or with a forest tree species, emphasizing the potential for production, as well as ecosystem services provisioning. Fruit trees are the primary driver of agroforestry adoption worldwide especially in the tropics (Wolz and DeLucia, 2018) and are considered as high value for agroforestry (Lauri *et al.*, 2019). Agroforestry and agroecology are two approaches of complex multifunctional systems, with agroforestry including explicitly woody plants and is considered that a relevant framework for agroecological practices (Wezel *et al.*, 2014). Although agroforestry considers mainly the structural and temporal arrangement of the system associated to different uses, agroecology clearly addresses the ecological-driven functioning of the system (Wezel *et al.*, 2014).

2. Potential of plurispecific agrosystems: the case of agroforestry

Agroforestry systems (AFS), as the intentional combination on one plot of trees and/or shrubs with crops or livestock, represent a good opportunity for agrosystem redesign (Dupraz and Liagre, 2008) and has been recognized as a sustainable agricultural practice for half a century (Garrity, 2012). Beneficial outcomes of agroforestry include reduced nutrient and pesticide runoff (Davis *et al.*, 2012), increased biocontrol (Gliessman, 1985), improved soil quality, erosion control, carbon sequestration (Cardinael *et al.*, 2017) and alleviate hazards linked to extreme climatic events (Leakey, 2014).

AFS, like other multi-species systems, are agrosystems designed to maximise resources (light, water, nutrients) usage in time and space by maximising positive interactions and minimising negative interactions (Jose *et al.*, 2004). Therefore, using different indicators such as the land equivalent ratio (LER), it is possible to compare mono-specific agrosystems and multi-specific ones. Nevertheless, if we aim to achieve higher yields in an agroforestry system, we need to understand what interactions take place and how they will influence plants growth. It is possible to achieve higher yields in an agroforestry system if the interspecific competition is lower that then intraspecific competition (Gliessman, 1985). However, the relations between the different components of the agrosystems will be modified as the plants are aging. To apprehend these complex systems, we can compartment interactions in aboveground and belowground interactions.

2.1. Impact of aboveground interactions among plants

Interactions in AFS depend on the species and the disposition of the trees. The interactions intensity will be different if the trees are on the border of the field or inter-cropped (Jose *et al.*, 2004). The most noticeable aboveground interaction is the competition for light between the species (Jose *et al.*, 2004) which causes plants to experience a modification in light quantity and quality because of light absorption by surrounding vegetation. The chlorophyll of neighbouring plants filters out the red (600–700 nm) and blue (400– 500 nm) wavelengths of the sunlight while reflecting and transmitting most of the far-red (FR) wavelengths (700–800 nm). In response to a drop in the red to far red ratio (R:FR) (Vandenbussche *et al.*, 2005), two major strategies have been recognized for maximizing fitness under shaded or partially shaded conditions (Henry and Aarssen, 1997; Gommers *et al.*, 2013). The first strategy, known in literature as the shade avoidance syndrome, consists in maximizing light interception through morphological and phenological traits that contributes to space occupation (Ballaré *et al.*, 1997). It includes traits as stem and petiole elongation, increased specific leaf area (ratio of leaf area to leaf dry weight), apical dominance, hyponasty, reduced branching and accelerated flowering (Smith and Whitelam, 1997; Foulkes *et al.*, 2010). The second strategy found in shade tolerant species aims at maximizing net carbon fixation through shade-adapted leaf physiology (Givnish, 1988). While shade and non-shade species optimize light capture and utilization through what is known as the carbon gain hypothesis (increased specific leaf area, increased photosystem II:I ratios and lower chlorophyll a:b ratios) (Givnish, 1988; Valladares and Niinemets, 2008), shade-tolerant species suppress shade avoidance traits (Niinemets and Valladares, 2004). However, shade adaptation and its effect on plant development have been mainly studied on annual plants and in a controlled environment and little is known of perennials response to shade (Matsubara, 2018; Maron, 2019).

Still, aboveground negative interactions can be limited by diminishing the density of trees, their disposition, precocity or growth period (Chirko *et al.*, 1996). However, the shade provided by the trees can also benefit the annual crops by limiting excessive summer radiations as stated before (Lin, 2007, 2011; Quinkenstein *et al.*, 2009). Trees will also impact the microclimate that can benefit the shaded plants depending on the climate. For example, the row of trees can act as a windbreaker that will affect the evapotranspiration demand and therefore improve the water use efficiency (Quinkenstein *et al.*, 2009). But a humid microclimate is also going to favour cryptogamic diseases (Gliessman, 1985) which can be a problem for apple trees because of *Venturia inaequalis* the pathogen responsible for apple scab if the selected cultivar is not resistant. The introduction of trees will also create new ecological niches by modifying the landscape that can offer new habitat that can contribute to increase the number and the diversification of natural enemies and pest (Jose *et al.*, 2004; Quinkenstein *et al.*, 2009).

2.2. Impact of belowground interactions among plants

Belowground interactions between perennial and annual plants depend of the spatial location of their roots. In an ideal situation where the roots of each different species are present in different compartments of the soil, competition will be less important than where there are in the same (Gliessman, 1985; Cardinael *et al.*, 2017).

Perennial plants usually have the majority of their fine roots in the first thirty centimetres of the soil and so are in competition with the annual crop (Jose *et al.*, 2006). However, most of the trees used in agroforestry have deep roots that will explore, if the depth of soil allows it, horizons of soils inaccessible to the annual plants (Rowe *et al.*, 1998; Jose *et al.*, 2001). Studies have shown that in agroforestry tree roots colonized deeper soil layers and were more vertically oriented (Cardinael *et al.*, 2015). Thereby, tree's roots can act as an interception net for the leached nutrients (Allen *et al.*, 2004). These nutrients will then be available to the annual plants after decomposition of the litter in the case of deciduous trees and if the leaves are left on the plot. In the same way, trees will also be able to absorb nutrients coming from the bedrock alteration (Schroth, 1995).

Deep roots could also act as a hydraulic lift if the top horizons are dryer than the bottom (Caldwell *et al.*, 1998; Jose *et al.*, 2004). If the quantity of water moved by this phenomenon is important enough, it could limit competition for water in mixed species systems. Even small amount can have a positive impact such as (i) making available nutrients that are not in a dry soil, (ii) facilitate root exploration and (iii) keeping roots active in a temporary dry soils and allow a quick recovery of activity (Pierret *et al.*, 2016). Furthermore, roots exploration can be improved thanks to the pores created by the tree's roots on one hand and biological activity improving soil structure on the other hand (Hulugalle and Lal, 1986).

Usually, tree roots occupy every soil horizon and thus are in competition with other plants for water and nutrients when they become a limiting factor. Even if this interaction can favour the separation of root systems (Pierret *et al.*, 2016), yield will be negatively impacted (Smith *et al.*, 1999) as the trees develop especially when they are still young (Parker and Meyer, 1996). A study has shown that walnut tree shallow roots in agroforestry grew mainly during the spring-summer period which could increase the competition with other plant (Germon *et al.*, 2016). Some species will also exudate allelochemicals in the rhizosphere that can harm the annual crop (Rizvi *et al.*, 1999). For example, apple trees have been reported to be sensitive to juglone, the phenolic compound that is the agent of *Juglans* spp. allelopathy (Galusha 1870; McWhorter *et al.*, 1874) cited in (Jose, 2011). Soil under 10-year-old black walnut trees (*Juglans nigra*) alley cropping system can have significant amounts of juglone if release rates are greater than the abiotic and microbial transformation rates (von Kiparski *et al.*, 2007). However, the concentration of juglone drops significantly with distance from the walnut tree row (Jose and Gillespie, 1998a) and the highest concentrations of juglone measured do not exceed the concentration inhibition threshold of crops typically considered for intercropping (Jose and Gillespie, 1998b). More recent studies showed that there are several processes that can be altered by lower concentration of juglone which can limit water and nutrient uptake (Hejl and Koster, 2004; Böhm *et al.*, 2006). In the light of this knowledge, it is safe to hypothesise that apple trees planted at 6 metres or more of walnut trees are probably not influenced by walnut allelopathic effect, but it could be a confounded factor for interpreting the effect on apple trees planted near walnut trees.

3. Prototyping fruit tree based agroforestry: the case of an apple orchard

Our study was developed on apples that is one of the most important fruit production of temperate climate and whose architecture and functioning has been extensively studied in conventional orchards (Volk, 2017). Apples are among the oldest and most important fruit crops in the world (Harris *et al.*, 2002). They have been cultivated since ancient times, in fact, archaeological studies have shown that they were cultivated already in 1000 BC (Juniper *et al.*, 1998).

Cultivated apples are a result of extensive ancient hybridization of various species of the genus *Malus* Mill., a member of the *Rosaceae* Juss. family, subfamily *Pomoideae* (pome fruits) (Jackson, 2003; Webster, 2005). Over hundred botanical names have been published for the cultivated apple (Qian *et al.*, 2010; Cornille *et al.*, 2012, 2014), however, *Malus domestica* Borkh. is now the correct binomial nomenclature for the cultivated apple (Qian *et al.*, 2010). Some morphological characteristics shared by apple cultivars in the world are: woolly pubescence on young stems and on the lower surface of the leaves, dull green leaves, elliptic-ovate in shape, with irregularly saw toothed margins, woolly pubescence on flower stalks and calyx, and pome fruits indented at the base with persistent calyx (Webster, 2005).

While interesting to reduce the negative impact of conventional orchard management agroforestry systems designed around fruit trees in temperate climate are poorly developed and studied and usually put the fruit tree in the upper strata (Lauri and Simon 2019) as typically illustrated in mixed fruit tree and vegetable farms that combine fruit trees and market gardening (Paut *et al.*, 2021). In tropical climate agroforestry designed around fruit trees (e.g. coffee and cocoa which are shade-adapted species) is a common practice. A recent study on coffee shows that light use efficiency increases with shade leaving net primary productivity fairly stable across all shade levels (Charbonnier *et al.*, 2017). Furthermore, shade has been proposed as a solution to improve tree water status and water use efficiency during drought periods (Nicolás *et al.*, 2005; Girona *et al.*, 2012). While light interception has been reported to be a primary factor to fruit yield (Palmer *et al.*, 2002) some studies have shown that under a moderate water stress net shading improved yield in apple (Lopez *et al.*, 2018). The benefit of shade was multi-factorial, it improved the tree water status, delayed fruit maturity hence giving more time for fruit growth and reduced photo-inhibition. Therefore, there is an incentive to study increasingly complex woody plant combination in temperate climate by combining timber trees, fruit trees and annual crops or shrubs on different strata (Lovell *et al.* 2018; Lauri *et al.* 2019).

Apple tree based agroforestry systems (AT-AFS) could be an interesting solution to reduce pesticide use since current high-density monoclonal orchards are usually highly susceptible to pests and diseases and, therefore, dependent on pesticides (Simon *et al.*, 2017). In addition to the aforementioned interests, an apple-based agroforestry systems could also be of interest in the Mediterranean area to limit the adverse effects of recurrent excessive summer radiation (light and temperature) which are responsible for annual field losses (Racsko and Schrader, 2012) and increase water use efficiency (Mupambi *et al.*, 2018). Indeed, these regions are regularly exposed to high solar irradiance and dry climate, even if competition for light and nutrients can affect fruit tree growth, the main expected benefits are related to the mitigation of microclimatic stresses (Lauri, Mézière, *et al.*, 2016). However, the inherent complexity of agroforestry systems is the primary hurdle to achieving their potential benefits. To optimize an agroforestry system, apart from selecting species with no allelopathic effects or strong interspecific competition, it is necessary to study them extensively to draw temporal and spatial assembling rules (Gliessman, 1985).

In our experiment apple trees are fertilized and irrigated according to the organic farming recommendations. As a result, we expect that aboveground competition (i.e. competition for light) will most probably be the limiting factor for the apple trees and we focused on the impact of shade of apple trees architecture, morphology, phenology and functioning.

3.1. Apple tree

Nowadays, apple trees are almost exclusively compound trees consisting of a scion grafted on a rootstock (Jackson, 2003; Tromp, Webster and Wertheim, 2005). Rootstocks are used to avoid juvenility, to control vegetative growth, to promote flower-bud formation, to improve cropping efficiency and quality of the fruits, and in some cases to provide winter hardiness and provide resistance or tolerance to some telluric diseases (Tromp, Webster and Wertheim, 2005; Hanke *et al.*, 2007).

The scion is the productive part of the tree that bears the different buds and two different type of shoots: (i) vegetative shoots and (ii) reproductive shoots composed of a flower cluster and one or more bourse shoots. All shoots are indeterminate in growth (Tromp, Webster and Wertheim, 2005) and emerge from buds which have the potential to produce leaf primordia only (vegetative shoots) or both leaf and flower primordia (reproductive shoots: Figure 1). Flower buds are found terminally on all types of shoots and terminally or axillary on long shoots after vegetative growth has stopped (Jackson, 2003; Tromp, Webster and Wertheim, 2005). Floral induction refers to the change of from the vegetative phase to the reproductive phase and occurs mainly in early summer, but it can be extended to early autumn under certain conditions. Floral initiation begins when the meristem flattens and continues as primordial sepals, petals, stamens and pistils form centripetally on the apex and grow into fully formed appendages (Pratt, 1988). Although flower induction can be inhibited by heavy cropping, some cultivars being notorious for their ‘biennial bearing’ habit, environmental factors also affect induction and initiation (i.e. solar radiation).

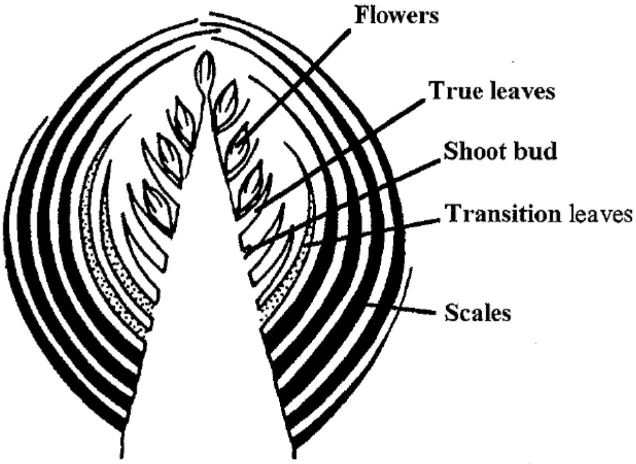


Figure 1: Apple flower (fruit) bud in diagrammatic longitudinal section, showing foliar appendages and flower buds (Abbot, 1970).

3.2. *How shade can affect apple tree*

Apple tree cultivars have all been selected and studied under optimal conditions, and their acclimation to different degrees of shade has mainly been studied under shade nets (Zibordi *et al.*, 2009; Morandi *et al.*, 2011; Bastías and Corelli-Grappadelli, 2012; Lopez *et al.*, 2018). While an alteration of leaf morpho-physiological traits (i.e. palisade thickness, stomatal aperture, and chlorophyll content) and an increased elongation is expected (Bastías *et al.*, 2012), little is known of the other architectural traits (i.e. number of ramifications, bud types) that apple trees will express in natural and fluctuating shade produced by upper trees and their adaptation to a changing environment. An important reduction in light intensity and at critical timing can affect apple production at different development stages leading to a decrease in fruit quantity and quality. The first negative consequence of shade on fruit production is the inhibition of floral initiation (Corelli-Grappadelli, 2003). Floral initiation is under the control of diverse environmental stimuli such as temperature and photoperiod and endogenous factors. The reason has not yet been fully elucidated as why shade reduces flower-bud initiation (Corelli-Grappadelli, 2003) but five genetically defined pathways have been identified that control flowering among which the photoperiod pathway refers to a regulation of flowering in response to day length and quality of light perceived (Srikanth and Schmid, 2011). The effects of shade nets on fruit growth development gave different results depending on climate and cultivars in relation to a reduction in light availability. In South Africa, for example, 20% shade nets reduced fruit growth for "Royal Gala" and "Cripp's Pink" (Gindaba and Midgley, 2005) and increase fruit growth for "Fuji" (Smit, 2007). Studies in Spain concluded that 20% shade nets did not affect fruit growth in "Mondial Gala" (Iglesias and Alegre, 2006). Furthermore, a reduction of light intensity in the period from 15 to 30 days after full bloom may greatly reduce fruit set (Byers *et al.*, 1985). During early stages of fruit growth, a decrease in photosynthesis and tree carbon assimilation (Zibordi *et al.*, 2009) can reduce fruit growth rates and induce fruit drops (McArtney *et al.*, 2004). Light availability can also affect fruit growth by affecting/changing carbohydrate partitioning between sinks (i.e. fruit and shoots). Shoots in full sun light are able to export photo-assimilates to fruit three weeks after full bloom while similar export for shaded shoots is reached only five weeks AFB for 70% of the shoots (Corelli-Grappadelli, 2003), suggesting that under shade shoot growth has priority over the fruit for photo-assimilate (Bepete and Lakso, 1998). Light quality also impacts fruit development, while shade has been reported to reduce fruit growth (Morandi *et al.*, 2011) another study reports an increase of maximal fruit growth up to 20% under blue shade nets that reduced the R:FR ratio and increased in the Blue:Red ratio (Bastías *et al.*, 2012).

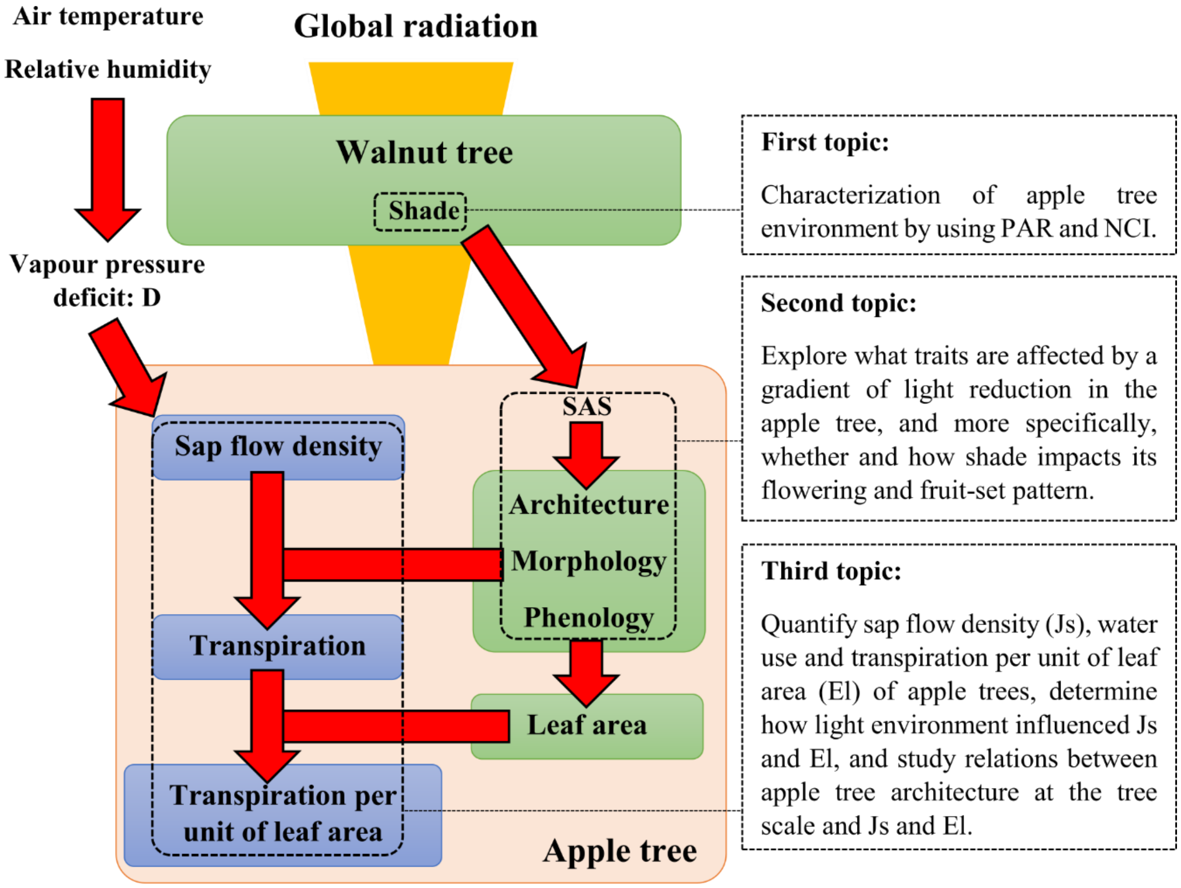
3.3. Tree architecture as a framework to analyse adaptation to agroforestry system

Tree architecture is a discipline of botany that was developed by Hallé (Halle and Oldeman, 1970). Combining four main criteria (i.e. growth and branching process, morphological differentiation of axes and position of reproductive organs) 23 architectural models were established (Barthélémy and Caraglio, 2007) each characterized by a unique combination of modalities off the four criteria. Apple tree combines features of two architectural models, Rauh and Scarrone. Branches are monopodial with lateral flowering in

Rauh and sympodial with terminal flowering in Scarrone. Apple trees combines both lateral and terminal flowering (Lauri and Laurens, 2005; Costes *et al.*, 2006). Based on the observation of tree shape and branching Lespinasse proposed ideotypes to describe the ’bearing habit’ of apple trees by combining the branching pattern and the distribution of fruiting (Lauri and Laurens, 2005). However, the variability of apple tree growth and fruiting patterns in each ideotype proved the limit of this approach so more detailed studies were developed on the behaviour of individual fruit-bearing shoots of various lengths with the objective to characterize the contribution of the fruit bearing shoot and the branch in bearing regularity (Lauri *et al.*, 1995, 1997) which led to three main results. First, cultivars are differentiated by the frequency of ‘extinction’ (i.e. axillary flower clusters whose bourse-shoots die) which showed that tree architecture also results from interactions between growing and non-growing organs (Lauri *et al.*, 2009). Second, cultivars can be deciphered by the frequency of bourse-over-bourse formation and third there is a positive correlation between bourse-over-bourse frequency and extinction. Upscaling these findings from the branch to the whole tree, three main strategies have been identified: (i) high bourse-over-bourse frequency indicates the ability to have a regular bearing pattern, (ii) low bourse-over-bourse could be related to irregular bearing if there is a synchronisation in shoots and (iii) lower bourse-over-bourse could be related to regular bearing if shoots are desynchronized (Lauri *et al.*, 1995, 1997; Lauri and Laurens, 2005). However, the actual bearing pattern of apple trees results both from its endogenous potential and the way it reacts to the training and pruning procedures (Breen, 2016).

4. Objectives

If apple tree based agroforestry systems are to be adopted by farmers in temperate climate, while having a high-value tree crop can lift some hurdles (Molnar *et al.*, 2013), many challenges remain and especially their capability to produce enough fruit in quantity and quality. To our knowledge, the expression of shade adaptation traits in response to a gradient of light and its effect on the architecture of fruit trees and interactions with flowering and fruiting and tree water use has yet to be studied. My study was carried out on young apples trees growing at various distances from large walnut trees for which the establishment of tree architecture and the entrance into flowering is crucial. My PhD research was developed around three main objectives (Figure 2).



First topic:

Characterization of apple tree environment by using PAR and NCI.

Second topic:

Explore what traits are affected by a gradient of light reduction in the apple tree, and more specifically, whether and how shade impacts its flowering and fruit-set pattern.

Third topic:

Quantify sap flow density (Js), water use and transpiration per unit of leaf area (El) of apple trees, determine how light environment influenced Js and El, and study relations between apple tree architecture at the tree scale and Js and El.

Figure 2: Conceptual framework around which the study was realised to estimate if and how an AT-AFS impacts apple trees development and water use (SAS: Shade avoidance/adaptation syndrome); PAR: Photosynthetically active radiation; NCI: neighbourhood crowding index).

The first objective was to characterize the environment of the apple trees. In the present study, a distance-dependent model of canopy crowding, hence referred to as neighbourhood crowding index (NCI), was developed to characterize the crowding of each apple tree without considering a specific limiting factor. However, in dense canopies in general (Huber *et al.*, 2020), and in agroforestry more specifically, light has been reported to be a critical factor for undergrown crops (Charbonnier *et al.*, 2017; Inurreta-Aguirre *et al.*, 2018). Quantifying the photosynthetically active radiation (PAR) reaching the crop is a prerequisite to understand the impact of shade trees (Charbonnier *et al.*, 2013) on plant physiology and architecture (Charbonnier *et al.*, 2017; Juchheim *et al.*, 2017). Different methods have been developed to estimate light regimes beneath top layer canopies using hemispherical photography (Bellow and Nair, 2003; Talbot and Dupraz, 2012; Dufour *et al.*, 2013; Schleppi and Paquette, 2017) or terrestrial scanners (Vincent and Harja, 2008; Vincent *et al.*, 2017; Zellweger *et al.*, 2019). The first part of this work starts by (i) comparing methodologies to estimate the quantity of PAR received by the target apple trees and (ii) estimate which of the

PAR, considered as the limiting factor, or NCI are better at explaining the variability of vegetative and reproductive traits measured on those apple trees. Our methodology included quantification of light received by the apple trees with hemispherical photographs and a terrestrial scanner as well as quantification of the size and distance to apple tree of all the neighbouring perennial plants that could influence the apple tree. Our hypothesis was that apple tree vigour is negatively correlated to an increasing number of neighbours and positively correlated to light quantity. Apple tree trunk cross section area was chosen as the first variable to compare the different indices developed to characterize apple trees since, according to Corner's rules, it is well correlated to other plant traits (i.e. number of ramifications) and can be correlated to plant overall biomass (Lauri, 2019). The leaf area or the number of flower clusters is also likely to be inversely correlated to the number of neighbours and positively correlated to the light quantity received.

The second objective was to determine (i) what are traits are affected by a gradient of light reduction in the apple tree, and (ii) more specifically, whether and how shade impacts its flowering and fruit-set pattern. Our methodology included quantification of morphological and architectural traits, at shoot and whole tree scales respectively, as well as phenological traits of flower clusters. As shade traits are complex and only partially documented on perennials, expected change in apple tree morphology (increased specific leaf area, elongation of internodes and axes), architecture (a decrease in the number of ramifications and flower clusters), and phenology (modification of flower clusters phenology and relative growth rate of shoots) will be specifically investigated here and discussed in the light of the knowledge on shade avoidant and tolerant annual species.

The third objective of this study was (i) to quantify sap flow density (J_s), water use and transpiration per unit of leaf area (E_l) of apple trees, (ii) determine how the light environment influenced J_s and E_l , and (iii) study relations between apple tree architecture at the tree scale and J_s or E_l. Summers in Mediterranean climate are characterised by excessive radiation and temperature which leads apple trees to close their stomata and therefore reduces photosynthesis rate (El-Sharkway and Hesketh, 1964). We hypothesised that apple trees planted near the walnut trees would not have their J_s or E_l impacted over the growing season. Benefiting from a favourable microclimate and less excessive radiation, we expect apple trees in shade to maintain their stomata open longer than apple trees in full light. Therefore, J_s during specific day, i.e. high vapour pressure deficit (D) should be higher for the apple trees in agroforestry compared to apple trees in full sun. Finally, since different degree of shade impact apple tree architecture (second objective; Pitchers *et al.*, 2021) we hypothesised that a more complex architecture could be related to an increase in apple tree E_l.

5. Materials and methods

5.1. Study site

The study site is located on the “Domaine de Restinclières” (Figure 3) in Prades-le-Lez, Hérault, France (43° 42' 12.168" N, 3° 51' 29.872" E - https://umr-system.cirad.fr/en/the-unit/research-and-training-platform-in-partnership/restinclieres-agroforestery-platform-rap). Apple trees (*Malus domestica* Borkh. cv. ‘Dalinette’ grafted on Geneva® G202 C.O.V. rootstock) have been planted as an intercrop in March 2016 on a plot with 10 rows of walnut trees (*Juglans nigra* x *Juglans regia* NG23 grown as timber) planted in February 1995 and a legume intercropped (*Medicago sativa* L.). Two rows of walnut trees are planted 13 meters apart. In 2007, the smallest walnut trees were thinned down in order to promote the bigger ones, leaving a gap of four meters between two walnut trees on the row, i.e. the initial distance between walnut trees within each row, or a multiple of four meters.

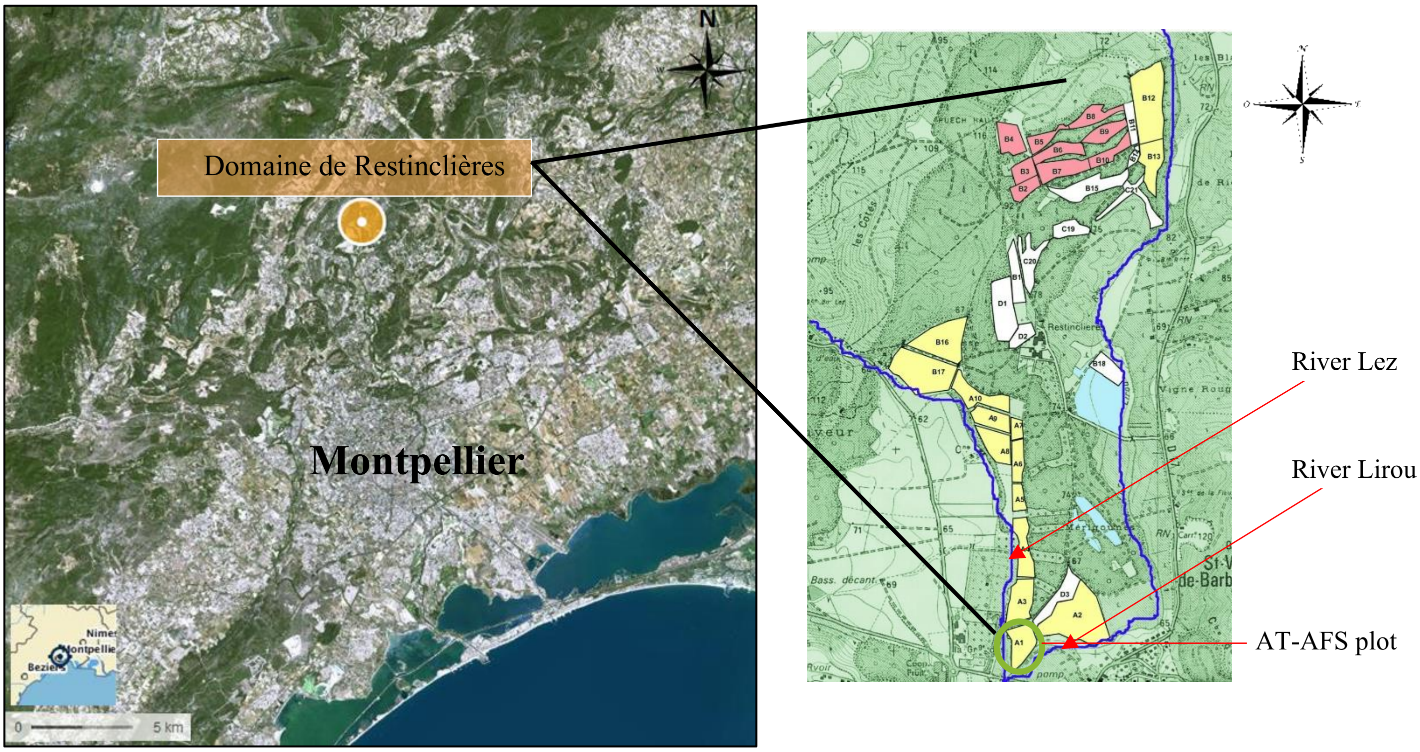


Figure 3: Geographic localisation of the “domaine de Restinclières” and the plot on which is located the apple – agroforestry experiment (AT-AFS) included in the GAFAM (Growing AgroForestry with Apple in the Mediterranean) project.

Apple trees were planted in three treatments: (i) full sun (agricultural control, AC), (ii) agroforestry on the inter row (AFIR), namely between two rows of walnut trees at 6.5 metres from each row, and (iii) agroforestry on the same row as walnut trees (AFR). In all cases, apple trees are distant of 1.3 m along the row with respect to the neighbouring apple tree or walnut tree depending on the treatment. Forty-five apple trees (fifteen in each treatment) were selected in 2017 depending on both their trunk cross section area (related to tree vigour) and light environment to obtain a gradient for these two variables in each treatment Figure 4. The trunk cross section area was measured with an electronic calliper at 60 centimetres above the ground and below the first branches. The light environment was estimated from hemispherical photographs took in 2018 on which a gap fraction analysis was performed.

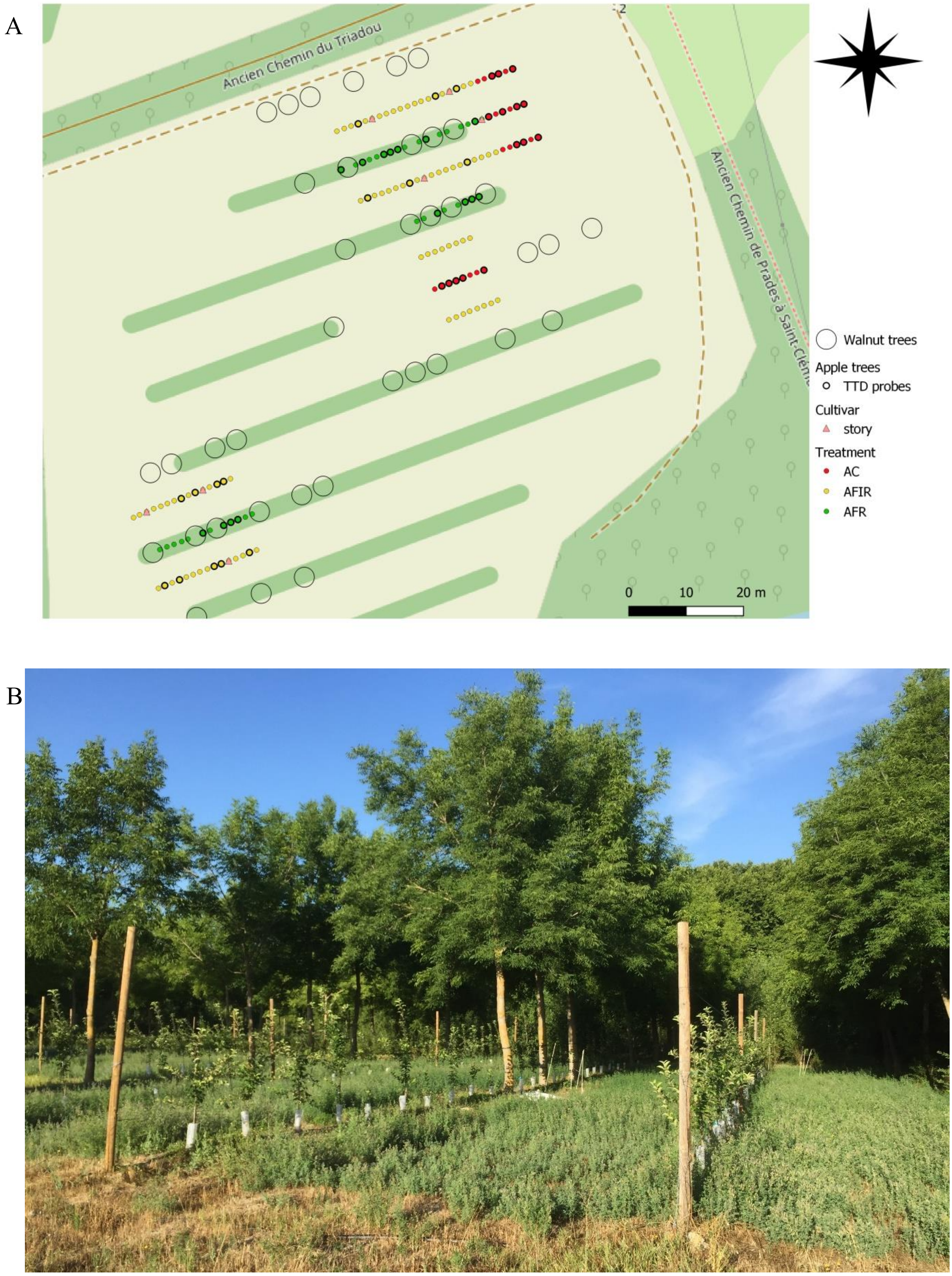


Figure 4: A. Experimental design located on “Restinclières Agroforestry Platform” in South-East France. The apple trees with a bold outer ring are the ‘dalinette’ trees on which all measurements were realized. B. Photograph of the experimental plot with walnut trees, apple trees and sainfoin.

Apple trees were managed according to organic growing guidelines. Woodchips were spread on apple and walnut-apple rows in order to control weeds and keep moisturing along the growing season. Drip irrigation and organic fertilization were provided to the apple trees following technical advice by an extension service. Apple trees were not pruned to let the natural architecture establish and limit the exogenous factors that could influence the trees response. Apple trees were partially thinned after full flowering in 2017 and 2018 to prevent the inhibition of floral initiation by gibberellins produced by seeds among other factors (Pratt, 1988; Wilkie *et al.*, 2008) and to limit competition between fruit sinks and promote vegetative growth.

Air humidity and temperature in weatherproof housing, and global and diffuse solar radiation were recorded every hour at a weather station located at 400 meters from our experimental fields with probes (Vaisala HUMICAP® by Campbell Scientific Inc. and BF5 Sunshine Sensor by Delta-T Devices; https://www.campbellsci.com/hmp35a). To check and adjust water irrigation, soil water content was measured at 3 different depths in the soil (20, 40 and 80 cm) under apple trees in the three plantation conditions by water content reflectometer (CS616, Campbell Scientific, Inc. Logan, UT, USA). All the probes were connected to a data logger (CR1000, Campbell Scientific, Inc. Logan, UT, USA).

5.2. Light quantification

In 2018, hemispherical pictures were taken with a camera (Sony NEX7-2381723 DSLRCompact-1289) equipped with a fish-eye lens (Lens.Cal Regent DSLR Compact, Regent Instrument Inc., https://www.regentinstruments.com/assets/winscanopy_system.html) above each apple tree. The 45 pictures were taken at dawn in approximately one hour before sunrise in diffuse light conditions, and at two walnut phenological stages, before budburst (minimum shade) and full foliation (maximum shade). Between these two dates a linear regression was used to estimate the quantity of light received each day by the apple trees. Images were then processed with the WinSCANOPY^{TM} software (Regent Instruments Inc.) to evaluate the quantity of photosynthetically active radiation (PAR) received by each of the 45 apple trees. Since this gap fraction analysis is based on thresholding of diffuse light to obtain a black (branches and leaves of walnut canopy above the apple trees) and white (sky) pixel map, there was no change on measurements during this one-hour time measurement.

In august 2018, i.e. at maximal walnut foliation, terrestrial laser scanning (TLS) was performed using the Riegl VZ-400 scanner operated at a pulse repetition frequency of 150 kHz with a maximum range of 950 m, and a pulse sampling interval of 0.04°. Scans were conducted with active short range detection, which means that the minimum measurement distance is around 1.5 m. Since the scanner has a field of view of 360°×100° in horizontal and vertical direction, we performed a horizontal and a 90° tilted scan at each scan location to cover the full sphere on 36 locations in our experimental plot. A dense scan pattern was chosen with an approximate distance between consecutive scan locations of 10–15 m. Reflective, cylindrical reference targets were placed on the plot which ensured that at least five common targets were measured from two proximal scan locations for geometric co-registration. For each TLS point cloud, the reference targets were mapped based on their high reflectivity of laser returns. The TLS scans were then co-registered in Riscan Pro (Riegl, v2.0.2) based on common reference targets among scans in 3D space and optimized to reduce the deviation among all targets. Plant area densities (PAD, m^2 m^{-3}) was then estimated using the AMAPVox software (v1.1.5) which runs on individual laser scan point clouds and allows to merge the resulting voxel grids to retrieve total plant area density in 3D (Vincent *et al.*, 2017). The resolution used to estimate PAD was 0.008 m^3. The PAR was then simulated from the 30th of July to the 23rd of September 2018, i.e. during the period of walnut tree full foliation.

PAR computed from hemispherical photographs and from terrestrial laser scanning are here after referred to as PAR_{HP} as PAR_{TLS}, respectively. PAR was estimated from data collected in 2018 only and considered equivalent in 2019 since the canopy formed by the 24- and 25-year-old walnut trees was well established and did not significantly change between these two successive years.

5.3. *Apple tree traits*

During 2018 and 2019, phenology of flower clusters was followed on the 45 apple trees. In 2018, thanks to the apple trees being young, all of the flower clusters were observed 27 times every two days from bud burst (beginning of April) to fruit-set (middle of June). After fruit drop, all the fruits were removed to limit competition for photo-assimilates between sinks. In 2019, the phenology of 10 flower clusters randomly chosen in terminal position on long shoots were observed until fruit-set, if available (some apple trees in low light had less than 10 flower clusters). The stages of the flower clusters were assessed using the Fleckinger chart (Fleckinger, 1964). The growth rate of 10 vegetative shoots per tree was monitored in 2019 every seven days until growth cessation, and relative growth rate (RGR, i.e. (shoot length at date 1 - shoot length at date 2) / shoot length at date 1) was computed.

At the end of each growing season the architecture of the 45 apple trees was analysed. Namely, each axes of the apple tree wer described considering two variables: its type (floral, vegetative or latent) and its position within the tree architecture (branching order with the trunk as order 1 and within each branch node rank of branching from the bottom). The diameter and length of the trunk, and all the axes more than 3 mm in diameter, were measured. In 2020, the number of flower clusters was assessed. Multiscale tree graph (MTG) was used to describe each tree structure and OpenAlea platform was used to extract the architectural variables at the tree scale (Pradal *et al.*, 2008, 2015). OpenAlea was used to get the number of each bud type for the 45 apple trees for each year as well as the number of new shoots and their length.

Tree leaf area was estimated in two steps. Firstly, in 2018, allometric equations between the length on an axis and true leaf area were computed on axes sampled from our experimental plot and depending on light environment, full sun and shaded trees. Two axis categories were considered, (i) vegetative shoots and (ii) bourse-shoots (Lauri and Trottier, 2004) with 30 fully grown axes in each category. True leaf area was measured by scanning all leaves of a shoot then analysing the images with WinFOLIA^{TM} (https://regent.qc.ca/assets/winfolia_about.html). Secondly, for both years, 2018 and 2019, inferred leaf area from the allometric relations was computed for each axis category of the described trees and summed up to get total leaf area of each tree.

5.4. Sap flow measurement

Granier-type single probes with a sensitive tip of 2 mm diameter and 8 mm length (UP GmbH, Cottbus, Germany) were used to measure the sap flow of 45 apple trees. The probes were inserted in the beginning of march 2018 at the base of the trunk, approximatively 20 cm above the graft point. The general TTD system with single probe was described in Do *et al.* 2011. The heating power was tuned at 0.08 W according to the ratio of 0.1 W per 10 mm of probe dissipation length. Our experiment applied a cycle of 10 min heating and 20 min cooling, which allowed a measurement of flux density every 30 min. The temperature signal was recorded before heating and at intermittent times (1, 2, 3, 4, 5, 10, 30, 60, 120, 300 and 600 seconds) in the heating phase via a data logger (CR1000, Campbell Scientific, Inc. Logan, UT, USA).

To convert the heat signal into a xylem sap flow a calibration was performed in the laboratory. Five branch sections of 20–40 mm in diameter, and 500mm in length from the same apple tree cultivar were selected. After being cut the surface of active xylem for each branch was measured with a calliper before immersing them in a plastic bag full of water and stored in a cold storage. The allometric relation between active xylem area and mean diameter was established on fifteen branches (*xylem area* (cm^2) = $0.0034 * \text{mean diameter}^{2.1664}$ (*mm*), $r^2 = 0.996$). Before installation, the stem was re-cut under fresh water at both ends to a final stem length of 400 mm. Then, two Granier-type single probes with a sensitive tip of 2mm diameter and 8mm length (UP GmbH, Cottbus, Germany) were inserted into the stem on opposite sides. The spacing was 100mm between probes, and between the probes and the ends of the cut-stems. The stem was maintained in a vertical position and connected to a peristaltic pump (model MS- REGLO, Ismatec SA, Glattbrugg-Zurich, Switzerland) in order to impose different flow rate. The pump was connected to a reservoir containing a KCl solution (20 mM) to prevent the development of microbes and safranine to check the active sap-wood area by staining. The flow rate was controlled by weighing the output with an automatic balance (0.01 g accuracy, Adventurer model, Ohaus, Parsippany, NJ, USA). The pump was managed in order to have a flux density at 0.5, 1, 3, 5 and 7 $L.dm^{-2}.h^{-1}$ with stabilized steps of 1 h. The thermal index expressed as K_l was then calculated. K_l is based on the ratio between the maximum temperature increase [recorded under zero flow (T_0)], and the increase under the measured flow (T_u) (Isarangkool Na Ayutthaya *et al.*, 2010; Do *et al.*, 2011).

$$K_l = (T_0 - T_u) / T_u \quad (1)$$

By correlating the thermal index with the J_s in $L.dm^{-2}.h^{-1}$, it was possible to determine a second degree polynomial (2).

$$J_s = -9.5923K_l^2 + 22.415K_l, \; r^2 = 0.95, \; RMSE = 29.4\% \quad (2)$$

SFD was then corrected depending on the temperature of the matrix using a methodology inspired by Flint *et al.* 2002. After testing the effect of the temperature at five different temperatures (4, 16, 22, 28 and 40°C) and for 4 different J_s (0, 1, 3 and 7 $L.dm^{-2}.h^{-1}$) a second order polynomial was fitted to a model of prediction of dimensionless slope (S).

$$S = -9e^{-05} * J_s^2 - 5e^{-05} * J_s - 0.0009, \; r^2 = 0.73 \quad (3)$$

Equation (3) was then used in an iterative process to correct the heat signal to a matrix temperature of 22°C: (i) an initial estimate of S was obtained by using the SFD obtained with equation (2), (ii) an estimate of the deviation was computed from the equation $d = 22 - S * T_b$ where T_b is the initial temperature measured before the heating and 22 the reference temperature and (iii) T_u^* was obtained from the equation $T_u^* = T_u + d$. T_u^* was then used in equation (1) to estimate a new SFD. This process was repeated 3 times until the variation of SFD was inferior to 0.1.

All measures that were performed on the apple trees are syntethised in Figure 5.

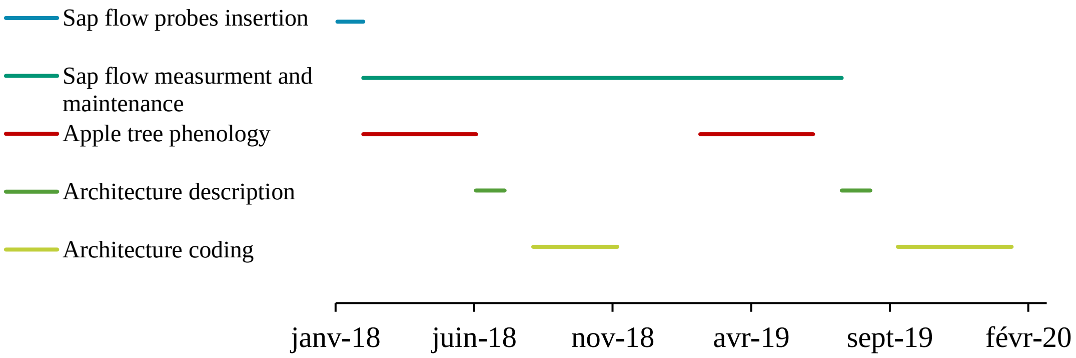


Figure 5: Timeline of all measurements that were performed on the apple trees during the two field years. Only the measurements that were used in my PhD are reported on this graph.

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CHAPTER 2: *A CRITICAL ASSESSMENT OF NEIGHBOURHOOD CROWDING INDEX: APPLICATION STUDY IN AN AGROFORESTRY SYSTEM OF TIMBER AND FRUIT TREES.*

Submitted to Applications in Plant sciences

Short Title for Running Head: Pitchers et al. - A critical assessment of NCI in an agroforestry system

A CRITICAL ASSESSMENT OF NEIGHBOURHOOD CROWDING INDEX: APPLICATION STUDY IN AN AGROFORESTRY SYSTEM OF TIMBER AND FRUIT TREES.

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ABSTRACT

- **PREMISE.** Working in multispecific agrosystem can be challenging with regard to the number of varying factors. This study focuses on characterizing the impact of neighborhood plants on apple tree growth and development in a multi-strata agroforestry system. Indicators based on quantifying a limiting factor, namely the quantity of photosynthetically active radiation (PAR) were compared to a distance-dependent neighbourhood crowding index (NCI).
- **METHODS.** After comparing two methodologies to quantify PAR, hemispherical photographs and terrestrial laser scanning, the target apple tree growth and flowering was analysed using PAR from each methodology and NCI.
- **RESULTS.** PAR estimated from the terrestrial scanner explained a higher proportion of the variability of the different measured traits, i.e. trunk cross section area, leaf area and the number of flower clusters, than PAR estimated from hemispherical photographs. Both methodologies always explained a higher proportion of these traits than NCI.
- **DISCUSSION.** Our results suggest that considering the limiting factor, light quantity in this study, in a managed agrosystem is more relevant than the NCI. Furthermore, having an indicator that integrates two factors, namely PAR and canopy volume computed from terrestrial laser scan, is more accurate.

Keywords: agroforestry, neighbourhood crowding index, light quantification, hemispherical photograph, terrestrial laser scanner, apple trees, walnut trees.

1. Introduction

Growing AgroForestry systems with Apple in Mediterranean climate (GAFAM) project looks at the growth and development of apple trees in a multi-strata agroforestry system where walnut trees (Juglans regia X Juglans nigra) planted in 1995 forms the upper stratum, apple trees (Malus domestica Borkh.) planted in 2016 the intermediate stratum and sainfoin (Onobrychis viciifolia) the herbaceous stratum (Pitchers *et al.*, 2020). Usually, the aim behind these experiments is to evaluate the capacity of a cultivation system to satisfy given objectives while factorial experiments are used to study and understand the effect of one or a few factors and their interactions taken in isolation, all other things being equal, on one or more dependant variables (Deytieux *et al.*, 2012). While the first approach aims to evaluate a global system with regard to defined objectives the second can help improve agrosystems by improving a technical aspect or a decision rule (Deytieux *et al.*, 2012). Analysing multi-species and multi-strata agrosystems is always challenging because of the heterogeneity and the number of factors that vary. When considering the PAR received we hypothesized that light would be a limiting factor and that, as stated in the Liebig limiting-factor paradigm, apple trees growth would be mainly constrained by light (Sinclair and Park, 1993). On the other hand, only considering the limiting factor to characterize plant growth in agrosystem goes back to ignoring the great flexibility of plants to acclimate morphologically and physiologically to changing environmental conditions (Sinclair and Park, 1993). NCI considered above and belowground interactions without considering that there is a prevailing compounds that would influence apple trees growth and development nor a specific limiting factor but that a number of resources will be simultaneously limiting apple trees growth.

Our study aimed at estimating the impact of increasing plant interactions in an agroforestry system on apple trees growth and development. Interactions in such systems depend on the species and the display of the trees, and vary depending on trees positioning in the plot (i.e. border or inter-cropped) and plantation density (Jose *et al.*, 2004). Interactions occur aboveground and belowground, with competition for light, and for water and nutrients, respectively (Jose *et al.*, 2004). Furthermore, species interactions at the neighbourhood level, hereafter referred to as crowding, are crucial to understand effects at the community level (Scherer-Lorenzen, 2014), because positive (facilitative) and negative (competitive) plant interactions emerge at small spatial scales (Stoll and Weiner, 2010). Forest ecologists have used different methods to study the effects of crowding on the growth of adult trees. The most common approach has consisted in testing the effects of distance-independent or distance-dependent measures of the local abundance of competitors on tree growth using regression models (Canham *et al.*, 2004).

In the present study, a distance-dependent model of canopy crowding, hence referred to as neighbourhood crowding index (NCI), was developed to characterize the crowding of each apple tree without considering a specific limiting factor. However, in dense canopies in general (Huber *et al.*, 2020), and in agroforestry more specifically, light has been reported to be a critical factor for undergrown crops (Charbonnier *et al.*, 2017; Inurreta-Aguirre *et al.*, 2018). Quantifying the photosynthetically active radiation (PAR) reaching the crop is a prerequisite to understand the impact of shade trees (Charbonnier *et al.*, 2013) on plant physiology and architecture (Charbonnier *et al.*, 2017; Juchheim *et al.*, 2017). Different methods have been developed to estimate light regimes beneath top layer canopies using hemispherical photography (Bellow and Nair, 2003; Talbot and Dupraz, 2012; Dufour *et al.*, 2013; Schleppi and Paquette, 2017) or terrestrial scanners (Vincent and Harja, 2008; Vincent *et al.*, 2017; Zellweger *et al.*, 2019).

The objectives of this study were to (i) compare methodologies to estimate the quantity of PAR received by the target apple trees and (ii) estimate which of the PAR, considered as the limiting factor, or NCI are better at explaining the variability of vegetative and reproductive traits measured on those apple trees. Our methodology included quantification of light received by the apple trees with hemispherical photographs and a terrestrial scanner as well as quantification of the size and distance to apple tree of all the neighbouring perennial plants that could influence the apple tree. Our hypothesis was that apple tree vigour is negatively correlated to an increasing number of neighbours and positively correlated to light quantity. Apple tree trunk cross section area was chosen as the first variable to compare the different indices developed to characterize apple trees since, according to Corner's rules, it is well correlated to other plant traits (i.e. number of ramifications) and can be correlated to plant overall biomass (Lauri, 2019). The leaf area or the number of flower clusters is also likely to be inversely correlated to the number of neighbours and positively correlated to the light quantity received.

2. Materials and methods

2.1. Study site and plant material

The study site is located on the in the Restinclières Agroforestry Platform (Prades-le-Lez, Hérault, France, 43° 42' 12.168" N, 3° 51' 29.872" E - https://umr-system.cirad.fr/en/the-unit/research-and-training-platform-in-partnership/restinclieres-agroforestery-platform-rap). Apple trees (Malus domestica Borkh. cv. 'Dalinette' grafted on Geneva® G202 C.O.V. rootstock) have been planted as an intercrop in March 2016 on a plot with walnut trees (Juglans nigra x Juglans regia NG23 grown as timber) planted in February 1995 and a legume intercropped (Medicago sativa L.). Two rows of walnut trees, oriented east/west, are 13 metres apart, with four metres between trees in the row and with possibly one or more walnut trees missing. Apple trees were planted in three treatments: (i) full sun (agricultural control, AC), (ii) agroforestry on the inter row (AFIR), namely between two rows of walnut trees at 6.5 metres from each row, and (iii) agroforestry on the same row as walnut trees (AFR). In all cases, apple trees are distant of 1.3 m along the row with respect to the neighbouring apple tree or walnut tree depending on the treatment.

Forty-five apple trees (fifteen in each treatment) were selected in 2017 depending on both their trunk cross section area and light environment to obtain a gradient for these two variables in each treatment. The trunk cross section area was measured with an electronic calliper at 60 centimetres above the ground and below the first branches. The light environment was estimated from hemispherical photographs took in 2018 on which a gap fraction analysis was performed.

2.2. Apple tree traits

At the end of 2018 and 2019 growing seasons, each new axis of the 45 apple tree was described considering its type (flower buds, vegetative or latent). The diameter and length of the trunk and all axes more than 3 mm in diameter were measured with an electronic calliper and a measuring tape.

Tree leaf area was estimated using allometric equations between the length on an axis and leaf area which were computed on axes sampled from our experimental plot and depending on light environment (full sun and shaded trees). Two categories of 30 axes were considered, vegetative, i.e. shoots growing from a vegetative bud, and bourse-shoot, i.e. the shoot branched on the side of the inflorescence (Lauri and Trottier, 2004). Leaf area was measured by scanning all leaves of a shoot and analysing the images with WinFOLIATM (Regent Instruments Inc.). Then, for both years, 2018 and 2019, inferred leaf area from allometric relations were computed for each axis category of the described trees and summed up to get total leaf area of each tree developed in these two consecutive years.

2.3. NCI Computation

In 2017, every apple trees and walnut trees were georeferenced with a high precision global positioning system. The experimental plot was then mapped on QGIS v3.6.3 GRASS (Figure 6). NCI is a classical distance-dependent crowding index (Canham *et al.*, 2004; Fichtner *et al.*, 2017; González de Andrés *et al.*, 2018) in which the target tree growth is analysed as a function of the sizes and distances to neighbouring trees. For i = 1, …, n neighbours within a maximum distance (r=15 metres) of the target tree in all directions, the net crowding effect of the neighbours on the target tree over a given period, from t0 to t, is given by equation [1].

$$[1]\quad \mathrm{NCI} = \sum_{ij=1}^{n} \frac{\mathrm{TCSA_{ij}}}{\mathrm{distance_{ij}}}$$

Where TCSA is the trunk cross section area in 2018 at breast height of the neighbour i of specie j. The maximum distance of 15 meters was chosen based on shade cast of walnut trees during the equinox and the distance usually used in other studies (Canham *et al.*, 2004; González de Andrés *et al.*, 2018).

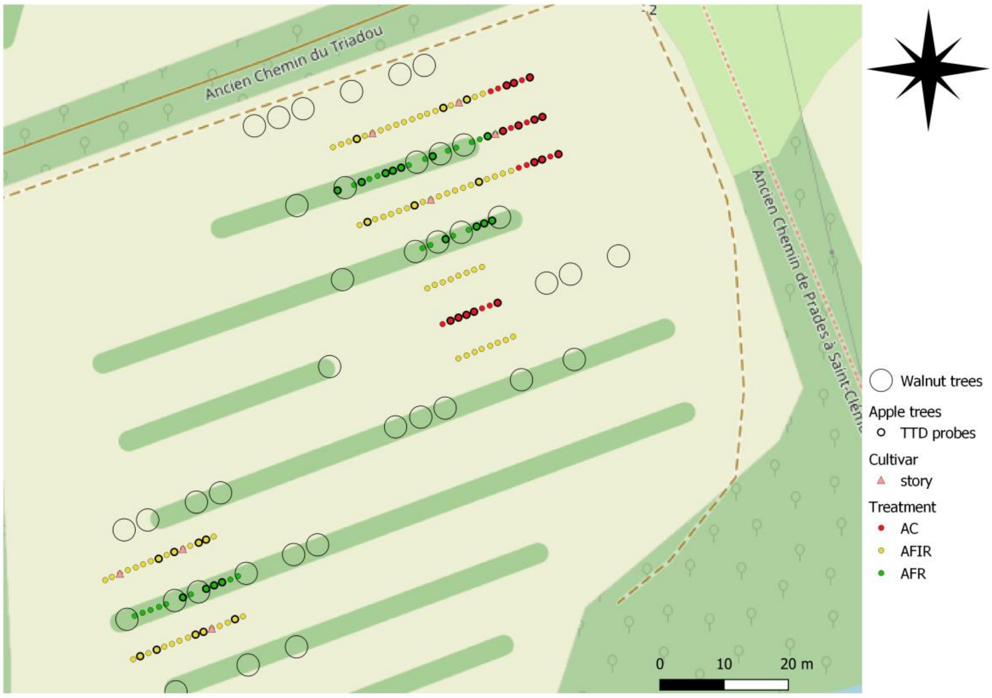

Figure 6: GAFAM experimental plot located on "Restinclières Agroforestry Platform" in South-East France. The apple trees with a bold outer ring are the 'dalinette' trees on which all measurements were realized.

2.4. Light quantification

In 2018, hemispherical pictures were taken with a camera (Sony NEX7-2381723 DSLRCompact-1289) equipped with a fish-eye lens (Lens.Cal Regent DSLR Compact, Regent Instrument Inc., https://www.regentinstruments.com/assets/winscanopy_system.html) above each apple tree. The 45 pictures were taken at dawn in approximately one hour before sunrise in diffuse light conditions, and at two walnut phenological stages, before budburst (minimum shade) and full foliation (maximum shade). Between these two dates a linear regression was used to estimate the quantity of light received each day by the apple trees. Images were then processed with the WinSCANOPYTM software (Regent Instruments Inc.) to evaluate the quantity of photosynthetically active radiation (PAR) received by each of the 45 apple trees. Since this gap fraction analysis is based on thresholding of diffuse light to obtain a black (branches and leaves of walnut canopy above the apple trees) and white (sky) pixel map, there was no change on measurements during this one-hour time measurement.

In 2018, terrestrial laser scanning (TLS) was performed using the Riegl VZ-400 scanner operated at a pulse repetition frequency of 150 kHz with a maximum range of 950 m, and a pulse sampling interval of 0.04°. Scans were conducted with active short range detection, which means that the minimum measurement distance is around 1.5 m. Since the scanner has a field of view of 360°×100° in horizontal and vertical direction, we performed a horizontal and a 90° tilted scan at each scan location to cover the full sphere on 36 locations in our experimental plot. A dense scan pattern was chosen with an approximate distance between consecutive scan locations of 10–15 m. Reflective, cylindrical reference targets were placed on the plot which ensured that at least five common targets were measured from two proximal scan locations for geometric co-registration. For each TLS point cloud, the reference targets were mapped based on their high reflectivity of laser returns. The TLS scans were then co-registered in Riscan Pro (Riegl, v2.0.2) based on common reference targets among scans in 3D space and optimized to reduce the deviation among all targets. Plant area densities (PAD, $m^2.m^{-3}$) was then estimated using the AMAPVox software (v1.1.5) which runs on individual laser scan point clouds and allows to merge the resulting voxel grids to retrieve total plant area density in 3D (Vincent *et al.*, 2017). The resolution used to estimate PAD was 0.008 m3. The PAR was then simulated from the 30th of July to the 23rd of September 2018, i.e. during the period of walnut tree full foliation.

PAR computed from hemispherical photographs and from terrestrial laser scanning are here after referred to as PAR_{HP} as PAR_{TLS}, respectively. PAR was estimated from data collected in 2018 only and considered equivalent in 2019 since the canopy formed by the 24- and 25-year old walnut trees was well established and did not significantly change between these two successive years.

2.5. Data analysis

Correlations and tests on slopes between PAR_{HP} and PAR_{TLS} were done with a standardised major axis approach (SMA) for each treatment (Warton *et al.*, 2012). Correlations were computed between independent variables, namely NCI, PAR_{HP} and PAR_{TLS} measured in 2018 and considered equivalent in 2019, and dependant variables measured on apple trees, namely, TCSA, leaf area and number of flower buds measured in both 2018 and 2019. To compare strength of correlations, fitting lines were adjusted on point clouds. Since the aim of this study was not to find the best fitting statistical relations but to compare different methodologies at explaining the variability of traits measured on apple trees only one parametric statistical law was chosen. Exponential fitting usually was the best fit compared to linear or power relations and was then applied to all correlations. As the PAR estimated is the same for both years (2018 and 2019) it is likely that while there is a causal relation between PAR and NCI with TCSA, LA and the number of flower clusters in 2019, it is a simple correlation for the number of flower clusters in 2018. Indeed, floral initiation usually occurs during the month of summer of the previous year under temperate climate in the northern hemisphere (Pratt, 1988).

All statistical analysis was done using Rstudio version 1.2.5001(Allaire, 2012) and with R (version 3.6.1) (Ihaka and Gentleman, 1996). Package Car (Fox *et al.*, 2012) was used for linear model regression and calculating nRMSE (normalized Root Mean Square Error) and nMBE (normalized Mean Bias Error), Smatr (Warton *et al.*, 2012) for SMA (Standardised Major Axis), and tydyverse (Wickham *et al.*, 2019) for data wrangling.

3. Results

3.1. Estimation of PAR under the canopy of walnut tree

To compare the two methodologies to estimate PAR reaching each of the 45 apple trees a correlation between PAR_{HP} and PAR_{TLS} was realized. PAR_{HP} explained 61% of the variability of PAR_{TLS} and there was no bias between the two methodologies ($r^2 = 0.61$, nRMSE = 0.47, nMBE = -0.02, Figure 7). When comparing the results for each treatment, the slopes between AFR and AFIR were not significantly different (t = 1.6, df = 2, P = 0.2) but the slopes between AC and AFIR (t = 18.9, df = 2, P = 1.3e-05), and AC and AFR (t = 33.7, df = 2, P = 6.4e-09) were significantly different.

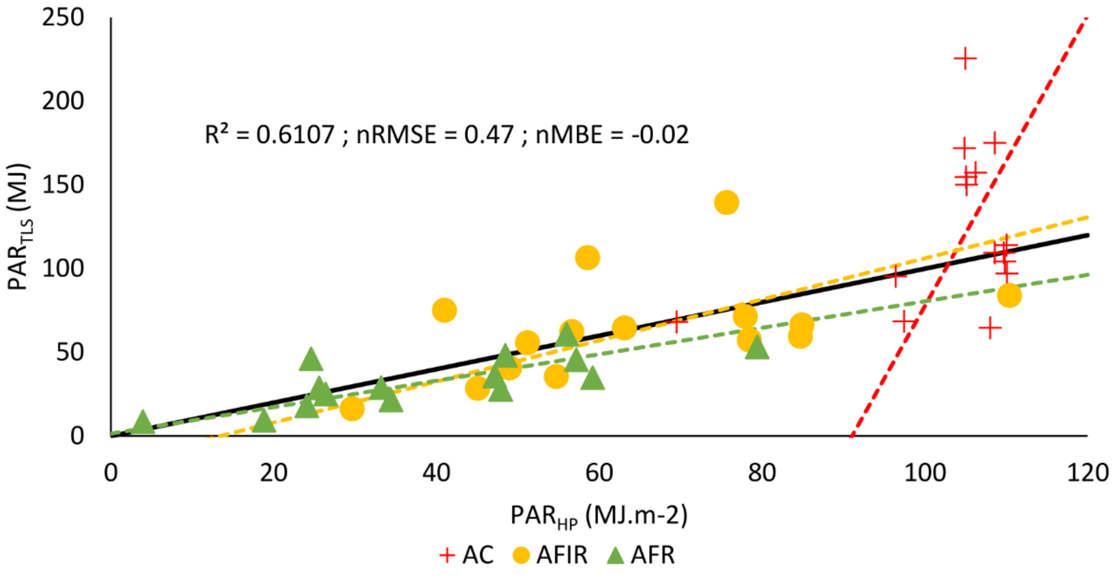

Figure 7: Correlation between PAR received in 2018 by the apple trees when in full shade (i.e. between the 30th of July and the 23rd of September) estimated with the terrestrial LiDAR (PAR_{TLS}) and the PAR estimated from the hemispherical photographs (PAR_{HP}) that are normalized to a square metre. Determination coefficient, nRMSE and nMBE were used to compare the two methodologies. The black curve is a 1:1 line and the dotted lines are the curve for each treatment estimated with a SMA approach. AC: Agricultural control; AFIR: agroforestry inter-row; AFR: agroforestry row.

3.2. Comparison between PAR_{TLS}, PAR_{HP} and NCI

PAR_{TLS} was better than PAR_{HP} at explaining the variability of TCSA of apple trees with 74% and 79% of explained variation in 2018 and 2018, respectively, against 63% and 60%, respectively, for PAR_{HP} (Figure 8A&B). NCI explained only 50 and 54% of the variability in TCSA in 2018 and 2019, respectively (Figure 8C).

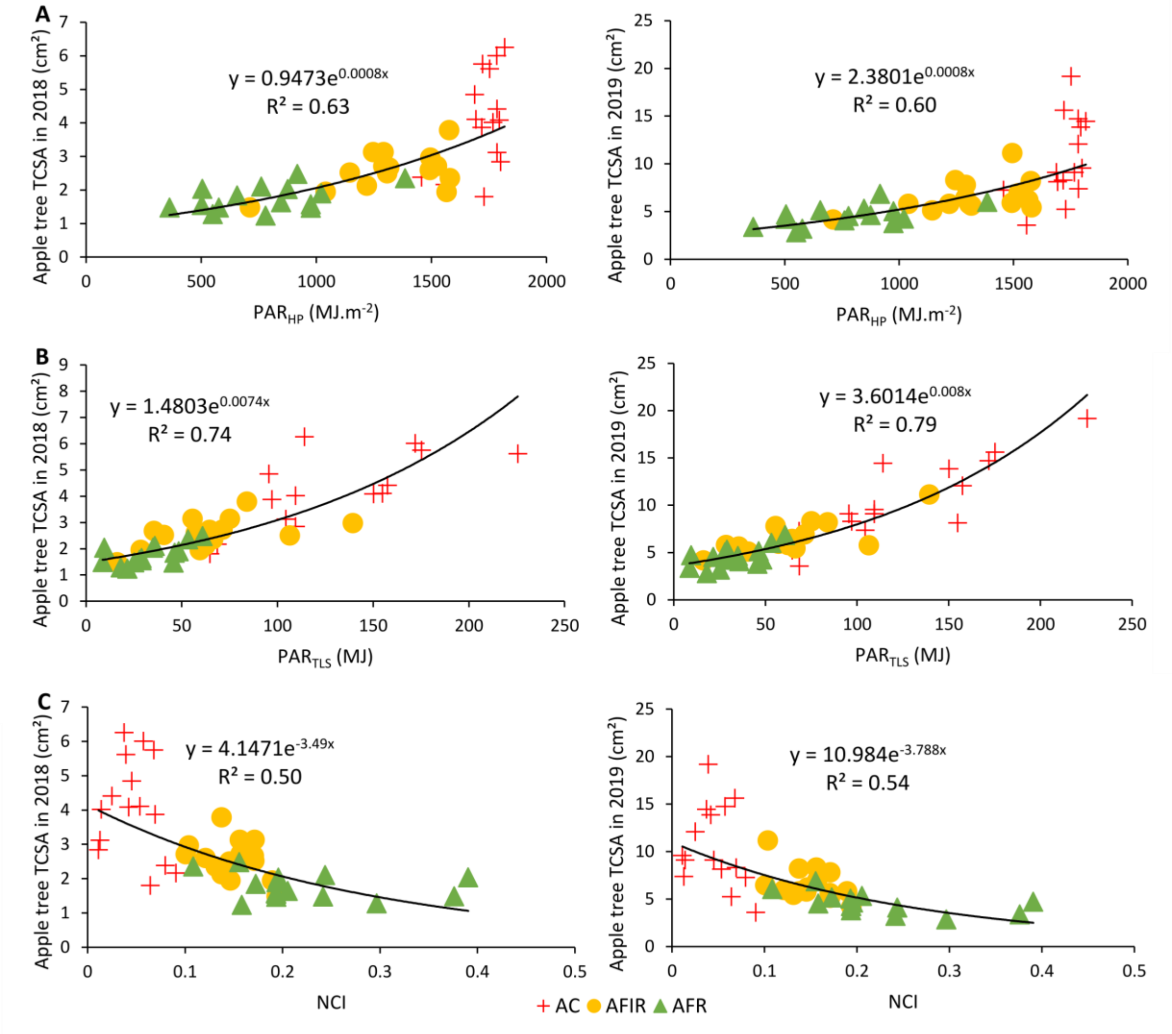

Figure 8: Correlation between apple trees trunk cross section area in 2018 and 2019 with (A) the PAR estimated from the hemispherical photographs (PARHP), (B) the PAR estimated from terrestrial laser scanning (PARTLS) and (C) the neighbourhood crowding index (NCI). All three environmental indicators were calculated in 2018.

To determine which environmental indicator, NCI, PAR_{TLS}, PAR_{HP} was the best indicator at explaining the impact of apple trees environment on their growth and development, correlations with leaf area and the number of flower clusters in 2018 and 2019 were performed (Figure 9). PAR_{TLS} was always the best indicator at explaining the variability of the considered traits except for the number of flower clusters in 2018 for which it was the worst indicator explaining only 45% of the variability. PAR_{HP} was also always better than NCI at explaining the variability of the considered traits. In the case of leaf area, PAR_{TLS}

explained 63% and 64% of the variability in 2018 and 2019, respectively, whereas PAR_{HP} explained 47% and 44% and NCI explained 36% and 38%. Furthermore, PAR_{TLS} explained 57% and 86% of the variability of the number flower clusters in 2018 and 2019 whereas PAR_{HP} explained 48% of the variability in both years and NCI 47% and 46%. Interestingly, the coefficient of variation for all morphological traits used to compare the three environmental indicators was higher for AC apple trees compared to the AFIR and AFR apple trees (Table 1). Inversely, the coefficient of variation for the number of flower clusters was higher for apple trees in agroforestry, except for AFIR in 2018, compared to apple trees in the agricultural control (Table 1). This could explain why graphically the three environmental indicators, NCI, PAR_{TLS} and PAR_{HP} explained a higher proportion of the variability for apple trees in agroforestry, both AFIR and AFR, compared to AC apple trees.

Table 1: Traits characterizing vegetative growth (Trunk cross section area (TCSA), Leaf area (LA)) and reproductive development (Number of flower clusters) of apple trees in each treatment (Agricultural control (AC), Agroforestry inter-row (AFIR), Agroforestry row (AFR)). Mean value ± sd, coefficient of variation (ratio of the standard deviation on the mean) between parentheses.

	Trunk cross section area in cm² (TCSA)		Leaf area (LA) in m²		Number of flower clusters	
	2018	2019	2018	2019	2018	2019
Agricultural control (AC)	4.1±1.4 (0.35)	10.5±4.3 (0.41)	2.24±0.71 (0.32)	3.71±1.42 (0.38)	75±58 (0.77)	224±137 (0.61)
Agroforestry inter-row (AFIR)	2.6±0.6 (0.22)	6.6±1.7 (0.26)	1.87±0.45 (0.24)	2.75±0.48 (0.29)	27±12 (0.45)	75±69 (0.92)
Agroforestry row (AFR)	1.8±0.4 (0.21)	4.5±1.1 (0.23)	1.32±0.28 (0.21)	2.05±0.48 (0.24)	10±11 (1.11)	20±19 (0.93)

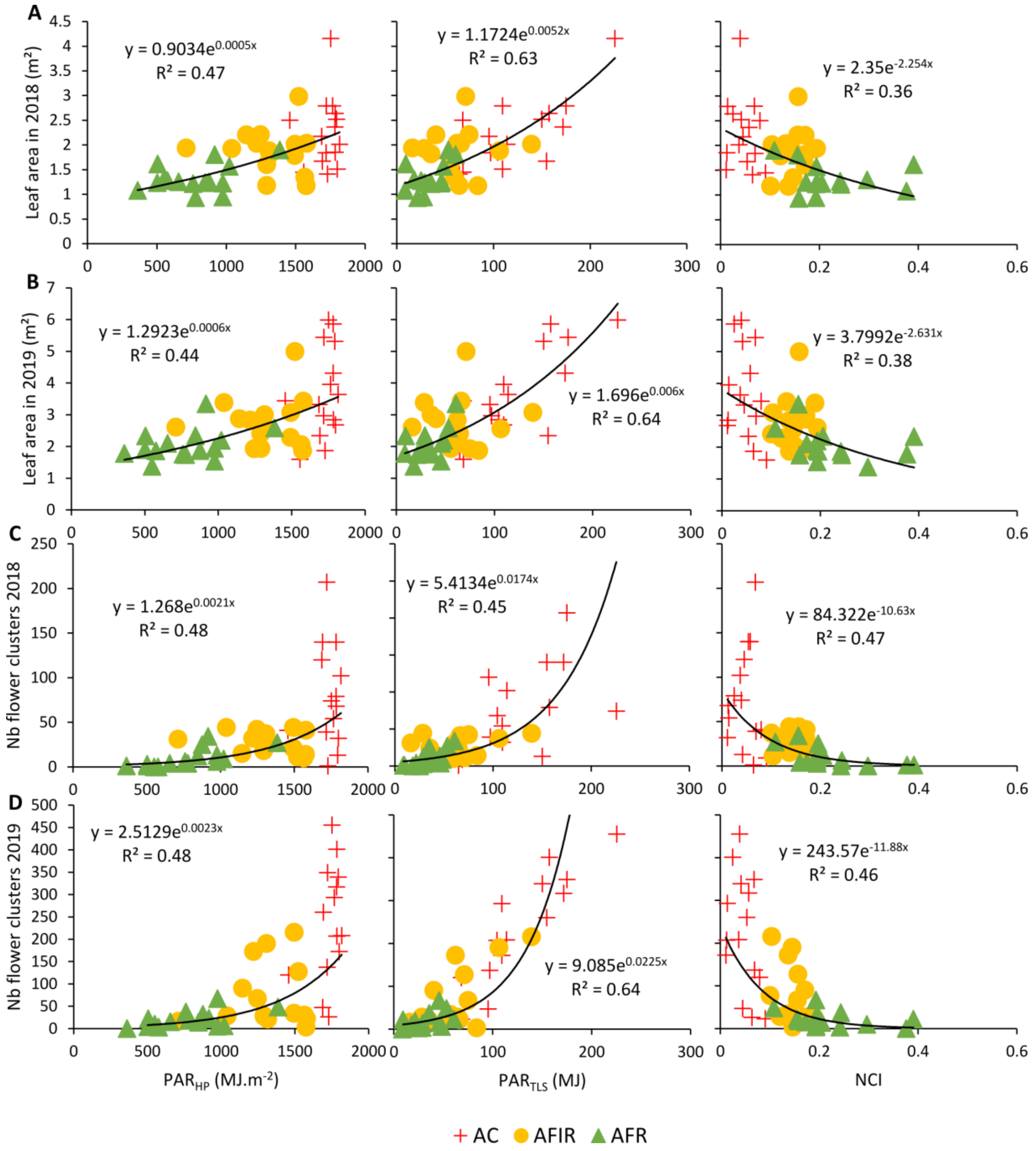

Figure 9: Graphical matrix of correlations between the different environmental indicators calculated in 2018 (PARHP, PARTLS, NCI) and measured variables that could be impacted by interactions with other plants. The proportion of variability in leaf area in 2018 (A) and 2019 (B) was tested as well as the number of flower clusters in 2018 (C) and 2019 (D).

4. Discussion

Our approach consisted in comparing three environmental indicators that would characterize the environment for each apple tree. Two of these indicators evaluating the PAR received during the growing season using hemispherical photographs and the PAR received during full foliation, i.e. when shade was maximum, using a terrestrial scanner only considered the aboveground interactions. When considering the PAR received, we hypothesized that light would be a limiting factor while NCI considered that there is no specific limiting factor but that a number of resources will be simultaneously limiting apple trees growth.

4.1. Comparison of PAR_{HP} and PAR_{TLS} methodologies

When comparing the two methodologies to estimate PAR on the same time period they were well correlated with no bias. However, when looking at apple trees in each treatment, the slope of the correlation for the apple trees in the agricultural control was significantly different from the slopes for the apple trees in agroforestry, AFIR and AFR. This suggests that while the two methodologies where accurate to evaluate the PAR under the canopy for apple trees in agroforestry it was different for apple trees planted in the agricultural control, i.e. in full light conditions. This variation was inherent to the methodologies themselves. The evaluation of PAR with the terrestrial scanner takes into account the volume of the canopy of apple tree and the light from all directions while the hemispherical pictures return the quantity of PAR normalized on a square metre (Vincent *et al.*, 2017). Consequently, for PAR_{HP} all apple trees in the control receive approximately the same quantity of light while for PAR_{TLS} apple trees absorbed different amounts of light. In this regard, the radiative balance estimated with a terrestrial scanner is more precise and encompasses more information when used with woody perennial plants likely related to the fact that it takes more into account light coming from the various directions which is known to have effects of plant growth and functioning (Zhang *et al.*, 2020).

Another information can be extracted from these correlations of PAR_{HP} and PAR_{TLS}. If the volume of the target tree occupies a space equivalent to a square metre than the apple tree is on a 1:1 line, an apple tree above the 1:1 line has a volume superior to a square metre and under the 1:1 inferior to a square metre. Apple trees in AFR are smaller than apple trees in AFIR which in turn are smaller than apple trees planted in AC. More precisely, apple trees planted on the same row as walnut trees had all a canopy volume inferior to a square metre except for one, apple trees planted on the inter-row had heterogeneous canopy volumes but globally around a square metre and apple trees planted in the control had in majority a canopy volume superior to a square metre. According to these estimations of PAR, apple tree growth and flowering seem to be diversely impeded in relation to the treatment which was confirmed when correlating the apple tree trunk cross section area to the different environmental indicators.

The methodologies used to quantify the limiting factor, light here, should be considered beforehand and chosen adequately depending on the study aims and resources. Using terrestrial LIDAR which permits to compute both tree canopy volume and intercepted PAR is more accurate and precise than using hemispherical photographs but comes at a higher cost and longer analysis. If working with hemispherical photographs, further analyses should estimate the area projected on the ground of each apple trees to improve environmental indicator.

4.2. Agrosystem management as key to control above- and belowground interactions

All three environmental indicators were promising at explaining the variability of different architectural and morphological traits, but the PAR estimated from the terrestrial scanner stood out. According to our results it appears that considering light was always better at explaining the variability of the different measured traits than NCI in 2018 and 2019. Our results confirmed that, in the specific context of our study, aboveground interactions were prevalent likely because of irrigation and fertilization that could minimize underground interactions if roots of apple trees and walnut trees occupy the same area for which we have no evidence. In other words, the management of the experimental plot participated in shifting the balance between above and belowground competition compared to a ‘natural’ ecosystem. Furthermore, NCI and PAR_{HP} determination coefficient were close to each other in 2018 and 2019 when looking at the number of flower clusters and the coefficient of variation for the number of flower clusters was usually lower for apple trees in AC. This suggests that the relation between the number of flower clusters and the quantity of light might not be as straightforward compared to TCSA or Leaf area and that a reduction in PAR increases the variability of the number of flower clusters. An indicator such as PAR_{TLS} which encompasses canopy volume is more robust at explaining growth and reproductive traits. Interestingly PAR_{TLS} always had a determination coefficient around 0.64 except for the number of flower cluster in 2018 while it only considered the radiative balance during full foliation, when competition for light is maximum. It would be possible to improve further PAR_{TLS} by scanning the plot before budburst and extrapolating PAR over the growing season. However, explaining 64% or more of the variability of measured traits might be enough for most of the study.

In the present study, the apple tree based agroforestry system is still young and the balance between above and belowground interactions might still shift especially when considering that drip irrigation will favour the presence of the root systems in the same area as neighbouring trees. In this case, an indicator that encompasses light, canopy volume and below ground competition could be more interesting and robust in the future.

Our study showed that when working in complex agroforestry systems using a continuous environmental indicator that helps characterize the interactions for each tree can complement analysis using only treatments as the independent factor because of the heterogeneity within a treatment. Distance-dependent crowding models like NCI has proven to be relevant to model tree architectural features but less than considering the limiting factor (i.e. light in this study) and quantifying it.

Beyond these considerations, and from an applied point of view, our study also brings some practical aspects that should be better taken in consideration to design optimized fruit-tree based agroforestry systems. Although our objective was not to search for the best fitting line between our environmental variables and vegetative and reproductive apple tree traits, all point clouds showed a disproportionate positive effect of PAR, and disproportionate negative effect of NCI, on TCSA, leaf area and number of flower clusters. In other words, and keeping on PAR only, a small increase of PAR in high PAR values increases more TCSA, leaf area and flowering than the same increase of PAR in low PAR values. This gives some minimal PAR values below which the target tree growth and flowering is considered as insufficient from an agronomic point of view. Looking at Fig.4, it appears that there is a negative impact of shade on floral initiation especially under 1500 MJ.m-2 or 150 MJ for PAR_{HP} and PAR_{TLS}, respectively. This suggests that under a certain threshold, approximatively a reduction of 30% of incoming PAR in our case, the capacity of apple trees to bear enough fruit is impeded. Considering that maximizing PAR interception is not always the best solution because of light damaging effects on leaf functioning and fruit quality (Grappadelli and Lakso, 2007) it is also likely that the effects of PAR on the three apple tree variables considered here are asymptotic instead of exponential. In this case, a maximal PAR interception threshold beyond which the target tree functioning is not improved or is even impeded should also be considered. Designing an apple tree based agroforestry system should then consider this optimal PAR values range, between minimal and maximal values, that should be determined more accurately depending on the genotypes.

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AUTHOR CONTRIBUTIONS

B. Pitchers conducted the investigation and led the writing. P-E Lauri and F.C. Do conceptualized and supervised the study as well as reviewed and edited the paper.

DATA AVAILABILITY

The data used in this research was published in the figshare.com data base (https://figshare.com/s/6b7ccc34b11a31fad044) and the digital object identifier (10.6084/m9.figshare.13242035).

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CHAPTER 3: *APPLE TREE ADAPTATION TO SHADE IN A FRUIT TREE BASED AGROFORESTRY SYSTEM*

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APPLE TREE ADAPTATION TO SHADE IN AGROFORESTRY – AN ARCHITECTURAL APPROACH

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Abstract:

- **Premise.** The expression of shade adaptation traits is expected to be stronger in low light conditions and can be detrimental to flowering and yield. Our study focused on the expression of shade adaptation traits of apple trees (Malus domestica Borkh cv. 'Dalinette') in an agroforestry system.
- **Methods.** The architecture of 45 apple trees in their third and fourth year was extensively described and analysed at the tree scale and compared depending on the light quantity received during the growing season. Flower cluster phenology and the relation between leaf area and floral initiation were also investigated.
- **Key results.** The number of growing shoots and the leaf area was reduced by shade even if specific leaf area increased with increasing shade. Shade did not modify primary growth but did decrease secondary growth. It made apple trees in shade slender, with a lower taper and also reduced the number and proportion of flower clusters. The correlation between floral initiation and leaf area was high both in full or moderate light but not for apple trees in low light. Shade did not impact the date of bud burst and the early phenological stages of flower clusters, but it reduced the number of days at full bloom.
- **Conclusions.** Our results suggest that while the architecture of apple trees is modified by a reduction in light intensity, it is not until a reduction of 65% that the capability to produce fruit is impeded. These results could help optimize the design of apple tree based agroforestry systems.

Keywords: shade, plurispecific agrosystems, plant architecture, floral initiation, shoot allometry, vegetativeshoots, floral phenology.

1. Introduction

In complex agrosystems, plants often experience a modification in light quantity and quality because of light absorption by surrounding vegetation. The chlorophyll of neighbouring plants filters out the red (600–700 nm) and blue (400– 500 nm) wavelengths of sunlight, while reflecting and transmitting most of the far-red (FR) wavelengths (700–800 nm). In response to a drop in the red to far-red ratio (R:FR) (Vandenbussche *et al.*, 2005), two major strategies have been recognized for maximizing fitness under shaded or partially shaded conditions (Henry and Aarssen, 1997; Gommers *et al.*, 2013). The first strategy, known in literature as the shade avoidance syndrome, consists in maximizing light interception through morphological and phenological traits which contributes to space occupation (Ballaré *et al.*, 1997; Huber *et al.*, 2020). It includes responses such as stem and petiole elongation, increased specific leaf area (leaf dry weight / leaf fresh area), apical dominance, hyponasty, reduced branching and accelerated flowering (Smith and Whitelam, 1997; Foulkes *et al.*, 2010). The second strategy, found in shade tolerant species, aims at maximizing net carbon fixation through shade-adapted leaf physiology (Givnish, 1988). While shade and non-shade species will optimize light capture and utilisation through what is known as the carbon gain hypothesis (increased specific leaf area, increased photosystem II:I ratios and lower chlorophyll a:b ratios) (Givnish, 1988; Valladares and Niinemets, 2008), shade-tolerant species suppress shade avoidance traits (Niinemets and Valladares, 2004). However, shade adaptation and its effect on plant development have been mainly studied on annual plants and in a controlled environment. Little is known of perennials response to shade (Matsubara, 2018; Maron, 2019).

Agroforestry, as the intentional combination of one plot of trees and/or shrubs with crops or livestock, has been recognized as a sustainable agricultural practice for half a century (Garrity, 2012). Beneficial outcomes of agroforestry include reduced nutrient and pesticide runoff (Davis *et al.*, 2012), increased biocontrol (Gliessman, 1985), improved soil quality, erosion control, carbon sequestration (Cardinael *et al.*, 2017). Agroforestry also alleviates hazards linked to extreme climatic events (Leakey, 2014). While intended to reduce the negative impact of orchard management, agroforestry systems designed around fruit trees in temperate climate are poorly developed and studied and usually put the fruit tree in the upper strata (Lauri and Simon, 2019). In tropical climates, the design of agroforestry systems integrating fruit trees, e.g. cocoa (Nijmeijer *et al.*, 2019), is a common practice. A recent study on coffee, shows that light use efficiency increases with shade, leaving net primary productivity fairly stable across various shade levels (Charbonnier *et al.*, 2017). Therefore, there is an incentive to study increasingly complex woody plant combinations in temperate climates by combining timber trees, fruit trees and annual crops or shrubs on different strata (Lovell *et al.*, 2018; Lauri *et al.*, 2019).

Our study was developed on the apple, one of the most important temperate climate fruit crop, whose architecture and functioning has been extensively studied in conventional orchards (Volk, 2017). Furthermore, apple tree based agroforestry systems, could be an interesting solution to reduce pesticide use since current high-density monoclonal orchards are usually highly susceptible to pests and diseases and are, therefore, dependent on pesticides (Simon *et al.*, 2017). In addition to the aforementioned interests apple based agroforestry systems could be of interest in the Mediterranean area to limit the adverse effects of recurrent excessive summer radiation (light and temperature) which is responsible for annual field losses (Racsko and Schrader, 2012). However, the inherent complexity of an agroforestry system is the primary hurdle to achieving its potential benefits. To optimize an agroforestry system, apart from selecting species with no allelopathic effects or strong interspecific competition, it is necessary to study them extensively to draw temporal and spatial assembling rules (Gliessman, 1985).

Apple tree cultivars have all been selected and studied under optimal conditions. Their acclimation to different degrees of shade has mainly been studied under shade nets (Zibordi *et al.*, 2009; Morandi *et al.*, 2011; Bastías and Corelli-Grappadelli, 2012; Lopez *et al.*, 2018; Mupambi *et al.*, 2018). Alteration of leaf morpho-physiological traits (i.e. palisade thickness, stomatal aperture, and chlorophyll content) and an increased shoot elongation is expected (Bastías *et al.*, 2012). However, little is known of the other architectural traits (i.e. number of ramification, bud types) that apple trees will express in natural and fluctuating shade produced by upper trees, or how apple trees will adapt to a changing environment. A significant reduction in light intensity and at critical point can affect apple production at different development stages, leading to a decrease in fruit quantity and quality. The first negative consequence of shade on fruit production is the inhibition of floral initiation (Corelli-Grappadelli, 2003). Floral initiation is under the control of diverse environmental stimuli such as temperature, photoperiod and endogenous factors. The reason has not yet been fully elucidated as to how shade reduces flower-bud initiation (Corelli-Grappadelli, 2003), however five genetically defined pathways have been identified that control flowering. One of them, the photoperiod pathway, refers to a regulation of flowering in response to day length and quality of light perceived (Srikanth and Schmid, 2011). The effects of shade nets on fruit growth development give different results depending on climate and cultivars in relation to a reduction in light availability. In South Africa, for example, 20% shade nets reduce fruit growth for ‘Royal Gala’ and ‘Cripp’s Pink’ (Gindaba and Midgley, 2005) and increase fruit growth for “Fuji” (Smit, 2007). Studies in Spain conclude that 20% shade nets do not affect fruit growth in “Mondial Gala” (Iglesias and Alegre, 2006). Furthermore, a reduction of light intensity in the period from 15 to 30 days after full bloom may greatly reduce fruit set (Byers *et al.*, 1985). During early stages of fruit growth, a decrease in photosynthesis and tree carbon assimilation (Zibordi *et al.*, 2009) can reduce fruit growth rate and induce fruit drop (McArtney *et al.*, 2004). Light availability can also affect fruit growth by affecting carbohydrate partitioning between sinks (i.e. fruit and shoots). Shoots in full sun light are able to export photo-assimilates to fruit three weeks after full bloom, while similar export for shaded shoots is reached only five weeks after full bloom for 70% of the shoots (Corelli-Grappadelli, 2003). This suggests that under shade, shoot-growth has priority over the fruit for photo-assimilate (Bepete and Lakso, 1998). Light quality also impacts fruit development, while shade has been reported to reduce fruit growth (Morandi *et al.*, 2011). Another study reports an increase of maximal fruit growth of up to 20% under blue shade nets that reduced the R:FR ratio and increased in the B:R ratio (Bastías *et al.*, 2012).

If fruit tree based agroforestry systems are to be adopted by farmers, while having a high-value tree crop can lift some hurdles for them (Molnar *et al.*, 2013), many challenges still remain and especially their capability to produce enough fruit in quantity and quality. To our knowledge, the expression of shade adaptation traits in response to a gradient of light and its effect on the architecture of fruit trees and interactions with flowering and fruiting has yet to be studied. Our study was carried out on young apples trees for which the establishment of tree architecture and the entrance into flowering is crucial. In this regard, the objectives of this study were to determine: (i) what are the traits that are affected by a gradient of light reduction in the apple tree, and (ii) more specifically, whether and how shade impacts its flowering and fruit-set pattern. Our methodology included quantification of morphological and architectural traits at shoot and whole tree scales respectively, as well as phenological traits of flower clusters. As shade traits are complex and only partially documented on perennials, expected change in apple tree morphology (increased specific leaf area, elongation of internodes and axes), architecture (a decrease in the number of ramification and flower clusters), and phenology (modification of flower clusters phenology and relative growth rate of shoots) will be specifically investigated here and discussed in the light of the knowledge on shade avoidant and tolerant annual species.

2. Materials and methods

2.1. Study site

The study site is located on the "Domaine de Restinclières" in Prades-le-Lez, Hérault, France (43° 42' 12.168" N, 3° 51' 29.872" E - https://umr-system.cirad.fr/en/the-unit/research-and-training-platform-in-partnership/restinclieres-agroforestery-platform-rap). Apple trees (*Malus domestica* Borkh. cv. 'Dalinette' grafted on Geneva® G202 C.O.V. rootstock) have been planted as an intercrop in March 2016 on a plot with 10 rows of walnut trees (*Juglans nigra* x *Juglans regia* NG23 grown as timber) planted in February 1995 and a legume intercropped (*Medicago sativa* L.). Two rows of walnut trees are planted 13 meters apart. In 2007, the smallest walnut trees were thinned down in order to promote the bigger ones, leaving a gap of four meters between two walnut trees on the row, i.e. the initial distance between walnut trees within each row, or a multiple of four meters.

Apple trees were planted in three treatments: (i) agricultural control (AC) (Figure 10A), (ii) agroforestry on the inter row (AFIR) (Figure 10B), namely between two rows of walnut trees at 6.5 meters from each row, and (iii) agroforestry on the same row as walnut trees (AFR) (Figure 10C). In all cases, apple trees were spaced at 1.3 m along the row from neighbouring apple or walnut trees depending on treatment. Rows of trees were oriented East/West.

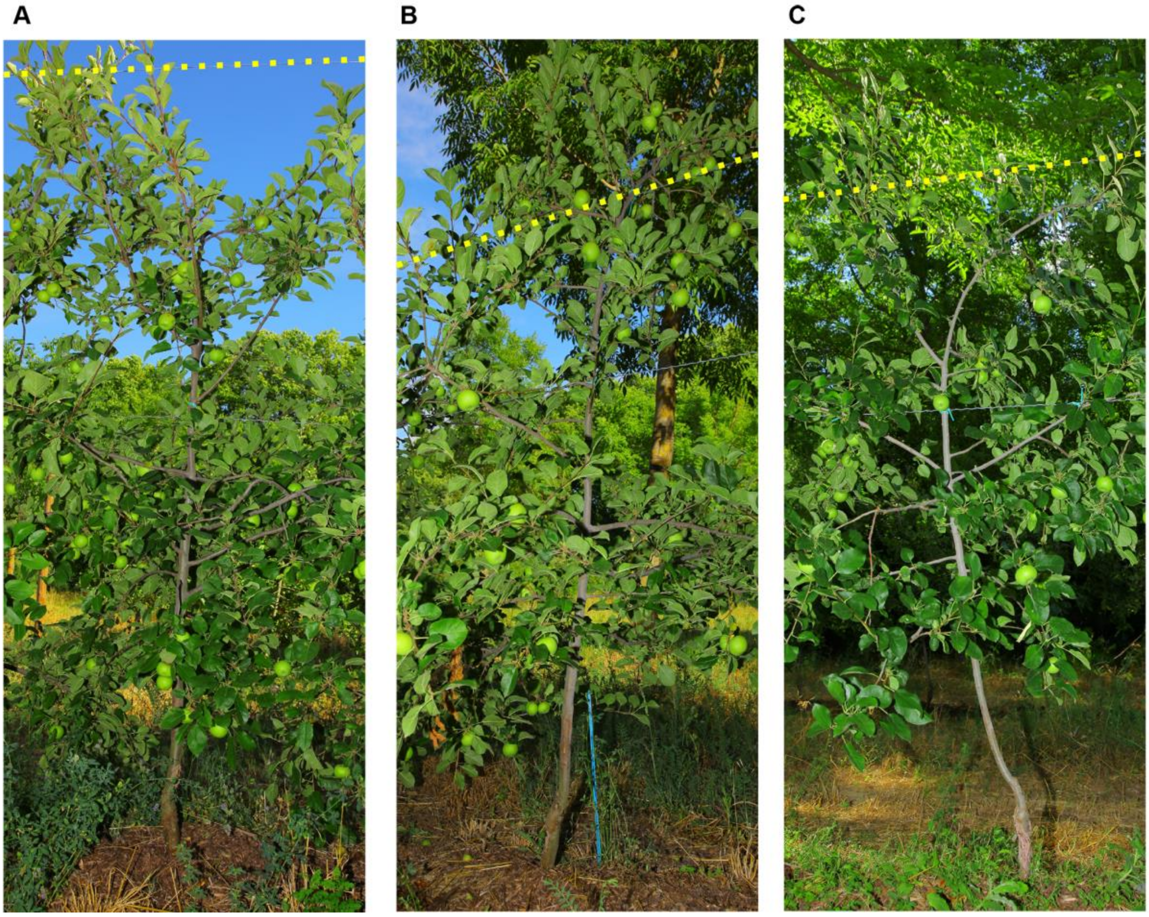

Figure 10: 4-year-old apple trees in full light in the agricultural control treatment (A), in moderate light on the inter-row of walnut trees (B) and in low light on the same row as walnut trees (C) from the experiment plot located at the “Domaine de Restinclières”. The dashed line represents a height of 2.1 meters.

Apple trees were managed according to organic growing guidelines. Woodchips were spread on apple and walnut-apple rows in order to control weeds. Drip irrigation and organic fertilization were provided to the apple trees following technical advice by an extension service. Apple trees were not pruned to let the natural architecture establish and limit the exogenous factors that could influence the trees response. Apple trees were partially thinned after full flowering in 2017 and 2018 to prevent the inhibition of floral initiation by gibberellins produced by seeds among other factors (Pratt, 1988; Wilkie *et al.*, 2008) and to limit competition between fruit sinks and promote vegetative growth.

Forty-five apple trees, fifteen in each treatment, were selected in 2018 depending on both their trunk cross section area and light environment to obtain a gradient for these two variables in each treatment. All the measurements were done in 2018 and 2019.

2.2. Light quantification

Hemispherical pictures were taken above each apple tree with a camera (Sony NEX7-2381723 DSLRCompact-1289) equipped with a fish-eye lens (Lens.Cal Regent DSLR Compact, Regent Instrument Inc., Québec, QC G1V 1V4, Canada, https://www.regentinstruments.com/assets/winscanopy_system.html). The 45 pictures were taken at dawn, one hour before sunrise, in diffuse light conditions, and at two walnut phenological stages, before budburst (minimum shade) and full foliation (maximum shade). Between these two dates, a linear regression was used to estimate the quantity of light received each day by the apple trees. Images were then processed with the WinSCANOPY^{TM} software (Regent Instruments Inc.) in order to evaluate the quantity of photosynthetically active radiation (PAR) received by each of the 45 apple trees. Since this gap fraction analysis is based on thresholding considering diffuse light, there was no change on measurements during this one-hour time measurement. A clustering approach was used to create three groups of apple trees according to the quantity of PAR received during the growing season, full light (1671 ± 120 MJ of PAR.m^{-2}), moderate light (1124 ± 182 MJ of PAR.m^{-2}) and low light (600 ± 138 MJ of PAR.m^{-2}). There were 21 apple trees in the full light group (15 from AC and 6 from AFIR), 15 in the moderate light group (8 from AFIR and 7 from AFR) and 9 in low light (1 from AFIR and 8 from AFR).

2.3. Data collection

During 2018 and 2019, phenology of flower clusters was followed on the 45 apple trees. In 2018, thanks to the apple trees being young, all of the flower clusters were observed 27 times every two days from bud burst (beginning of April) to fruit-set (middle of June). After fruit drop, all the fruits were removed to limit competition for photo-assimilates between sinks. In 2019, the phenology of 10 flower clusters randomly chosen in terminal position on long shoots were observed until fruit-set, if available (some apple trees in low light had less than 10 flower clusters). The stages of the flower clusters were assessed using the Fleckinger chart (Fleckinger, 1964). The growth rate of 10 vegetative shoots per tree was monitored in 2019 every 7 days until growth cessation, and relative growth rate (RGR; (shoot length at date 1 - shoot length at date 2) / shoot length at date 1) was computed.

At the end of each growing season the architecture of the 45 apple trees was analysed. Namely, each axis of the apple tree was described considering two variables: its type (floral, vegetative or latent) and its position within the tree architecture (branching order with the trunks as order 1 and within each branch node rank of branching from the bottom). The diameter and length of the trunk, and all the branches more than 3 mm in diameter, were measured. In 2020, the number of flower clusters was assessed. Multiscale tree graph (MTG) was used to describe each tree structure and OpenAlea platform was used to extract the architectural variables at the tree scale (Pradal *et al.*, 2008, 2015). OpenAlea was used to get the number of each bud type for the 45 apple trees for each year as well as the number of new shoots and their length. Shoots that were longer than 5 cm were considered long shoots, and shoots shorter or equal to 5 cm were considered short shoots in further analysis.

Tree leaf area was estimated in two steps. Firstly, in 2018, allometric equations between the length on an axis and true leaf area were computed on axes sampled from our experimental plot and depending on light environment, full sun and shaded trees. Two axis categories were considered, (i) vegetative, i.e. shoots growing from a vegetative bud, and (ii) bourse-shoot, i.e. the shoot branched on the side of the inflorescence (Lauri and Trottier, 2004) with 30 fully grown axes in each category. True leaf area was measured by scanning all leaves of a shoot then analysing the images with WinFOLIA^{TM} (https://regent.qc.ca/assets/winfolia_about.html). Secondly, for both years, 2018 and 2019, inferred leaf area from the allometric relations was computed for each axis category of the described trees and summed up to get total leaf area of each tree.

2.4. Data analysis

Different indicators were calculated to decipher the impact of light conditions on apple tree morphology. Slenderness (trunk height / trunk mean diameter) helps evaluate the elongation of the trunk and taper its conicity ((sub-terminal mean diameter – basal mean diameter) / trunk height). The proportion of flower clusters was calculated for each year as the number of flower clusters on one apple tree divided by the total number of buds (i.e. flower cluster, vegetative and latent).

Since the assumptions for ANOVA were very rarely met, all mean comparison tests (i.e. table 1 and Figure 2) were realised with a Kruskall–Wallis non parametric test by rank at each time point. The false discovery rate (Benjamini and Hochberg, 1995) was used for multiple significance testing and adjusting the p-value.. The fruit set rate, as the ratio of flower clusters with at least one fruit set on the total number of flower clusters (Lauri *et al.*, 1996) was analysed using a generalized linear mixed model with the apple tree as a random effect.

As floral initiation occurs the year preceding actual flowering (Pratt, 1988) correlations between the number of flower clusters in 2019 and the leaf area in 2018, and the number of flower clusters in 2020 and the leaf area in 2019, were done with a standardised major axis approach (SMA) for each group (Warton *et al.*, 2012). Allometric relationship between these two variables, leaf area one year and flowering the next year, was also tested when transformed with the log10 function (Warton *et al.*, 2006; Lauri *et al.*, 2014). When the slope is equal to 1 it indicates an isometric relation (i.e. proportional whatever the values) between the two variables. A slope different from 1 indicates an allometric relation between the two variables. Furthermore, a test on the slope, elevation and shift along the common slope was then performed to compare fitting between the groups.

The phenology of flower clusters from bud-burst to fruit-set was analysed using an ordered logistic regression. For each time point, flower cluster's phenology was compared depending on the light intensity received by the apple trees. The ordinal logistic regression model was parametrised as $logit(P(Y \leq j)) = \alpha_j - \beta_i X_i$ with *j* the phenological stages of the flower clusters and *i* the light treatment.

All statistical analysis were done using Rstudio (version 1.2.5001; Allaire, 2012) and with R (R core team, 2020). Packages agricolae (de Mendiburu and de Mendiburu, 2020), car (Fox *et al.*, 2012) and multcomp (Hothorn *et al.*, 2016) for non-parametric mean comparison, Smatr (Warton *et al.*, 2012) for SMA, Mass (Ripley *et al.*, 2013) for ordered logistic regression with the glmmPQL function to take into account overdispersion with a quasibinomial distribution, tydyverse (Wickham *et al.*, 2019) for data wrangling and ggplot2 (Wickham *et al.*, 2016) for graphics.

3. Results

3.1. Shade adaptation traits

As light quantity received by the apple trees decreased, there was no difference in total trunk height in 2019 but there was a significant decrease in trunk cross section area (Table 2). This resulted in a difference in slenderness and taper (Table 2). Slenderness was significantly higher in moderate and low light compared to full light. Taper, however, was significantly higher in full light than in moderate and low light. There were no statistically significant differences in the internode length looking at vegetative shoots and bourse shoots together or independently. Specific leaf area was affected by light intensity, with leaves being in full sun having a significantly lower specific leaf area than leaves in moderate and low light (Table 2).

Table 2: Summary of plant traits associated with the shade adaptation at organ (morphology) and tree (architecture) scales. Values are mean±SD. Kruskall Wallis rank test followed by Tukey's multiple comparison test were performed. In a same line, different alphabetical letters denote significantly different means at $P < 0.05$ ($df = 2$). As RGR was significantly impacted by light intensity at one-time point, only the results on this day are reported.

	Full light	**Moderate light**	**Low light**
Number of apple trees	21	15	9
PAR received (MJ/m^2)	1671 ± 120 ^{a}	1124 ± 182 ^{b}	600 ± 138 ^{c}
Axis and leaf morphology			
Trunk height in 2019 (cm)	286.4 ± 56.0 ^{a}	279.6 ± 38.5 ^{a}	252.5 ± 30.4 ^{a}
Trunk Section Area (cm^2)			
2018	3.7 ± 0.3 ^{a}	2.3 ± 0.1 ^{b}	1.61 ± 0.1 ^{c}
2019	9.6 ± 0.9 ^{a}	5.8 ± 0.3 ^{b}	4 ± 0.3 ^{c}
Trunk slenderness (m.cm^{-1})	1.40 ± 0.21 ^{b}	1.68 ± 0.14 ^{a}	1.80 ± 0.31 ^{a}
Trunk taper (mm.m^{-1})	9.29 ± 1.58 ^{a}	7.38 ± 1.42 ^{b}	6.44 ± 0.95 ^{b}
Internode length (cm)			
All shoots	1.56 ± 0.40 ^{a}	1.66 ± 0.45 ^{a}	1.69 ± 0.46 ^{a}
Bourse shoots	1.61 ± 0.42 ^{a}	1.69 ± 0.42 ^{a}	1.70 ± 0.51 ^{a}
Vegetative shoots	1.51 ± 0.37 ^{a}	1.62 ± 0.49 ^{a}	1.67 ± 0.42 ^{a}
Specific leaf area (cm^2.g^{-1})	149.4 ± 10.5 ^{b}	164.5 ± 12.1 ^{a}	170.3 ± 10.6 ^{a}
Tree Architecture			
Number of new shoots			
2018	192 ± 70 ^{a}	131 ± 18 ^{b}	89 ± 14 ^{c}
Long shoots (>5cm)	62 ± 27 ^{a} (33%)	46 ± 10 ^{ab} (35%)	33 ± 9 ^{b} (37%)
Short shoots (≤5cm)	130 ± 59 ^{a} (67%)	85 ± 33 ^{ab} (65%)	56 ± 12 ^{b} (63%)
2019	306 ± 138 ^{a}	189 ± 50 ^{b}	121 ± 22 ^{c}
Long shoots (>5cm)	128 ± 61 ^{a} (42%)	77 ± 20 ^{b} (41%)	49 ± 7 ^{c} (41%)
Short shoots (≤5cm)	178 ± 81 ^{a} (58%)	113 ± 34 ^{b} (59%)	73 ± 17 ^{b} (59%)
Leaf area (m^2)			
2018	2.16 ± 0.68 ^{a}	1.67 ± 0.39 ^{ab}	1.23 ± 0.18 ^{b}
2019	3.53 ± 1.31 ^{a}	2.44 ± 0.60 ^{b}	1.86 ± 0.26 ^{c}
Growth dynamic (24th May 2019)			
Relative growth rate (mm.cm$^{-1)}$	0.21 ± 0.14 ^{a}	0.16 ± 0.16 ^{b}	0.16 ± 0.14 ^{b}
Bourse shoots	0.18 ± 0.14 ^{a}	0.13 ± 0.12 ^{ab}	0.11 ± 0.13 ^{b}
Vegetative shoots	0.23 ± 0.14 ^{a}	0.20 ± 0.19 ^{a}	0.20 ± 0.13 ^{a}

There were significant differences in the number of new shoots in 2018 and 2019 for all light intensities. In both years, the number of ramifications decreased with light intensity, while the proportion of long and short shoots across the three light intensities remained roughly the same (Table 2). There was a difference in the leaf area in 2018 and 2019 (Table 2). In 2018, leaf area was significantly higher in full light compared with a low light environment. Leaf area in the moderate light environment was not significantly different from the other two light intensitiess. In 2019, leaf area was significantly different for each group and decreased with light intensity once again.

Shoot relative growth rate (RGR) was never significantly impacted by light intensity except for one day (May 24th 2019), 5 weeks after full bloom (Table 2). On this day, RGR in full light was significantly higher compared to a moderate and low light environment. However, looking at the RGR for the two different shoot types, vegetative and bourse shoots, there was no significant differences for vegetative shoots but RGR for bourse shoots in full light was significantly higher than for bourse shoots in low light.

3.2. Floral initiation

Although there was a strong increase in both the number and the proportion (Figure 11A) of flower clusters in trees between 2018 and 2019, the effect of light intensity was similar with a significant decrease of flower clusters initiation in decreasing light (Figure 11A). In 2018, there was a significant difference in the number and the proportion of flower clusters between the apple trees in full light and moderate light versus the trees in low light. In 2019, there was a significant difference in the number of flower clusters between apple trees in full light and trees in moderate on the one hand and low light on the other hand. However, looking at the proportion of flower clusters, apple trees in full light had a significantly higher proportion of flower clusters than apple trees in low light. Apple trees in moderate light were not significantly different from the other two groups. In 2018, there was no effect of light conditions on fruit set rate but in 2019 fruit set rate was significantly and inversely correlated to light conditions (Figure 11B).

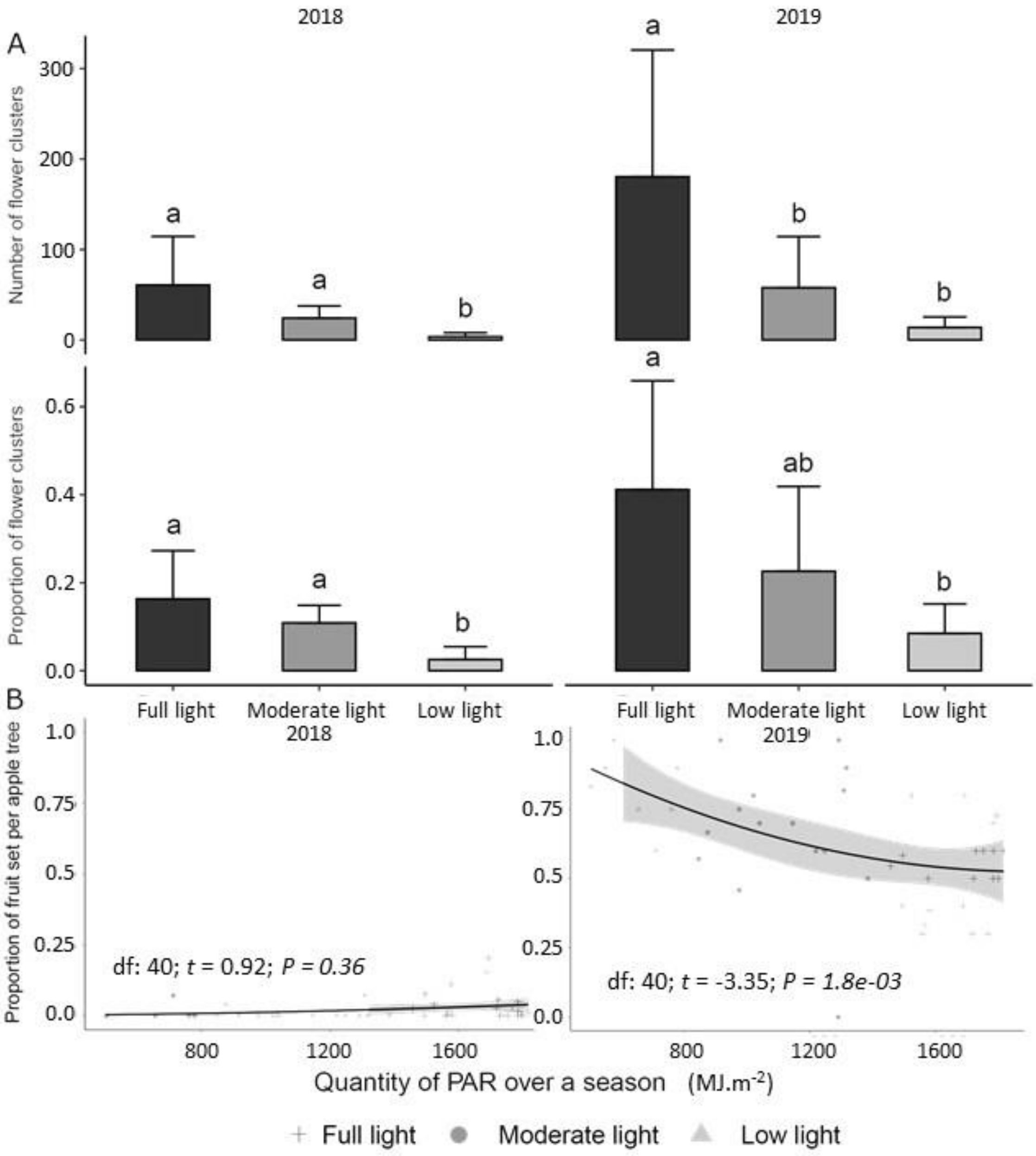

Figure 11: Number and proportion of flower clusters in 2018 and 2019 of apple trees in different light intensities (A). Data are means+SD. Kruskall Wallis rank test were performed to decipher significant differences followed by Tukey's multiple comparison test on the number of flower cluster in 2018 ($\chi2$ = 20.46, df = 2, P = 3.6e-05), the number of flower clusters in 2019 ($\chi2$ = 18.52, df = 2, P = 9.5e-05), the proportion of flower clusters in 2018 ($\chi2$ = 17.34, df = 2, P = 1.7e-04) and the proportion of flower in 2019 ($\chi2$ = 13.41, df = 2, P = 1.2e-03). (B) Plot of fruit set rate in relation to light quantity for 2018 and 2019. The relation was analysed with a GLMM with apple trees as a random effect.

The number of flower clusters of a current year was positively correlated with leaf area of the precedent year for both years (Figure 12). In full light, the correlation was highly significant for both years. 50% of the variability of the number of flower clusters in 2019 was explained by tree leaf area in 2018 and increased to 71% in 2020 when correlated by tree leaf area in 2019. In moderate light, the correlation was also significant for both years but the apple tree leaf area of the previous year only explained 37% of the variability of the number of flower clusters in 2019 and 31% in 2020. In low light, the correlation between the apple tree leaf area in the previous year and the number of flower clusters in the current year was not significant in both years. Comparing the curves between full light and moderate light, there was no difference in the slope or the elevation but there was a significant difference in the shift along the common slope for both years (Figure 12). Looking at the value of the slope in 2018 (Figure 12A), the slopes for both full light and low light were different from 1. As these curves were fitted in log transformed data it means there was a positive (slope superior to 1) allometric relation between the apple tree leaf area of the previous year and the number of flower clusters in current year. However, in 2019, the slope for full light and moderate light was not significantly different from 1, suggesting that this time around there was an isometric relation between the apple tree leaf area of the previous year and the number of flower cluster in the current year.

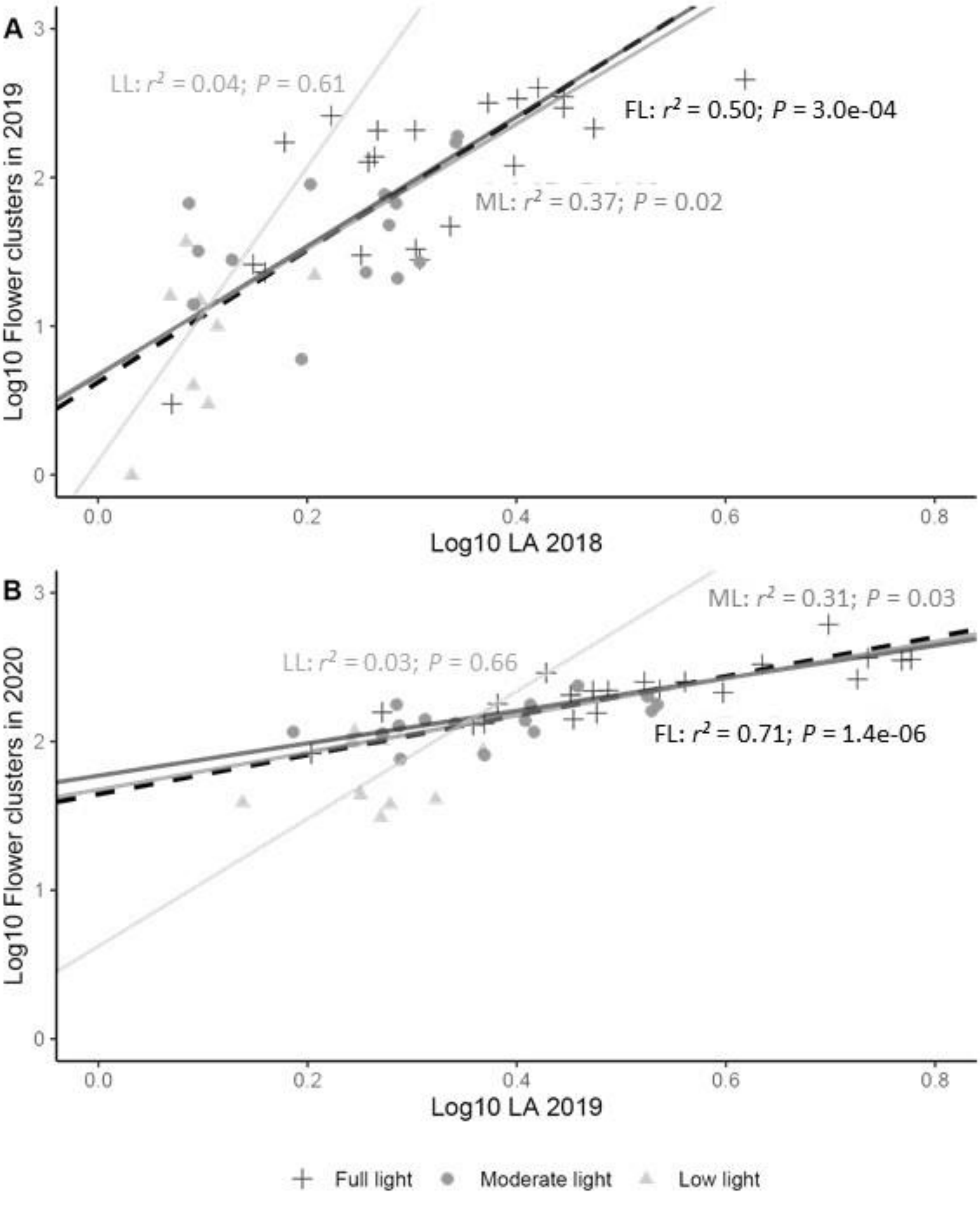

Figure 12: Correlation between the number of flower clusters in 2019 (A) and 2020 (B) and leaf area in 2018 and 2019, respectively. Data were log transformed to meet the normal distribution assumption. The lines were fitted using a SMA regression. The dashed line represents the common slope. Slopes were equal in 2019 (t = 0.02, df = 2, P = 0.9) and 2020 (t = 0.25, df = 2, P = 0.62) between full light and moderate light. The correlation being not significant in low light the comparison tests were not reported. A. Test on slope between FL and ML: Elevation: t = 3e-03, df = 1, P = 0.95; Shift: t = 7.96, df = 1, P = 4.7e-03 / Allometry test (slope different from 1): FL: r = 0.95, df = 19, P = 8.4e-11; ML: r = 0.93, df = 13, P = 6.1e-07. B. Test on slope between FL and ML: Elevation: t = 0.35, df = 1, P = 0.55; Shift: t = 12.67, df = 1, P = 3.7e-04 / Allometry test (slope different from 1): FL: r = 0.33, df = 19, P = 0.14; ML: r = 0.3, df = 13, P = 0.29.

3.3. Phenology

On one occasion, in 2018, there was a significant difference in flower cluster's phenology between full light and moderate light (Figure 13A). Flower clusters phenology was significantly more advanced in moderate light 37 days after budburst compared to flower clusters in full light (logit coefficient greater than one). It corresponded to phenological stages G and H according to the Fleckinger chart. Petal fall occurred faster in moderate light. For flower buds in low light, there was a significant difference compared to full light 32 days after budburst (Figure 13A). It corresponded to phenological stages G and H. Petal fall occurred precociously and faster for flower clusters in low light compared to full light. There were no statistical significant differences in flower cluster's phenological stages between moderate and low light.

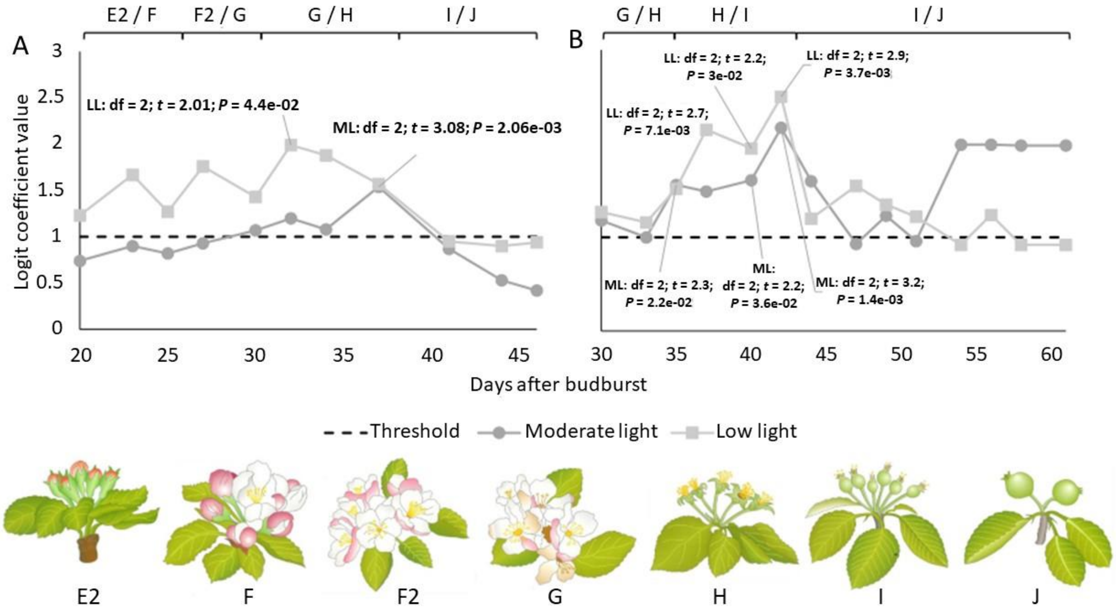

Figure 13: Flower clusters phenology affected by the light intensity in 2018 and 2019. Ordered logistic regression analyses were made comparing the flower phenology relative advance of a light intensity treatment has against another light intensity treatment. Only the significant comparisons are represented: "moderate vs full light" and "low vs full light". A logit coefficient higher than one means that apple trees floral clusters phenological stages in the group (i.e. moderate or low light) are more advanced than in the reference (i.e. full light). A logit coefficient lower than one means that the apple trees flower clusters phenological stages are less advanced compared to the reference. The letters above indicates the main phenological stages, according to Fleckinger chart, of the apple trees at these dates (E2: Petals can be seen, F: King flower opens, F: Full bloom, G: Start of petal fall, H: End of petal fall, I: Fruit set, J: Fruit growth).

In 2019, there was a significant difference in flower cluster's phenology between full light and moderate light 35, 40 and 42 days after budburst (Figure 13B). The phenological stages concerned were H and I which correspond to petal fall and fruit set. Once again petal fall occurred faster in moderate light. For flower buds in low light there was a significant difference in flower cluster's phenology 37, 40 and 42 days after budburst (Figure 13B). It concerned the same phenological stages, H and I. Petal fall occurred faster but not as precociously as in 2018.

4. Discussion

It is likely that light is only one factor among others that could act in these complex environments. For example, apple trees have been reported to be sensitive to juglone, the phenolic compound that is the agent of *Juglans* spp. allelopathy (Galusha 1870; McWhorter *et al.*, 1874) cited in (Jose, 2011). Soil under 10-year-old black walnut trees (*Juglans nigra*) alley cropping system can have significant amounts of juglone if release rates are greater than the abiotic and microbial transformation rates (von Kiparski *et al.*, 2007). However, the concentration of juglone drops significantly with distance from the walnut tree row (Jose and Gillespie, 1998a) and the highest concentrations of juglone measured do not exceed the concentration inhibition threshold of crops typically considered for intercropping (Jose and Gillespie, 1998b). A more recent study showed that there are several processes that can be altered by lower concentration of juglone which can limit water and nutrient uptake (Hejl and Koster, 2004; Böhm *et al.*, 2006). In the light of this knowledge, it is safe to hypothesise that apple trees planted on the agroforestry inter-row and the control are probably not influenced by walnut allelopathic effect, but it could be a confounded factor for walnut and apple trees planted on the same row.

Our study revealed that apple trees not only expressed shade adaptation for vegetative traits, usually observed in shade avoidance species, but also traits that were unexpected and had not been studied in perennial plants to our knowledge. The expression of these traits had an impact on the tree trunk morphology, architecture and capacity to bear fruits. The number of ramifications was positively correlated to light quantity received by the tree. However, noticeably, the proportion between long and short shoots stayed the same on the second and third year after plantation. This not only impacted the apple tree leaf area but also the proportion of flower clusters, which ultimately could impede the tree capacity to produce fruits. In the following, we discuss our results from two points of view, changes in morphology, architecture and phenology, and changes in the reproductive strategies which are tightly associated to vegetative growth.

4.1. Changes in morphology, architecture and phenology

Common traits observed in shade avoidance syndrome were observed in our study. As shown here, reducing light intensity decreased branching in the two studied years confirming the phenomenon of increased apical dominance under shade (Smith and Whitelam, 1997) observed on ‘Ginger Gold’ cultivar (Miller *et al.*, 2015). As expected, apple tree specific leaf area was higher in shade (Gommers *et al.*, 2013; Ballaré and Pierik, 2017) which is an adaptation to maximise carbon gain per unit of leaf mass (Evans and Poorter, 2001). In one study on coffee, an increase in specific leaf area was shown to be enough to compensate the reduction of photosynthetic rate due to light reduction and maintain a net primary production (Charbonnier *et al.*, 2017). However, in our study, changes in specific leaf area were not enough to compensate for light reduction as leaf area and trunk section area were significantly reduced. Photosynthetic rate and autotrophic respiration were not measured here but these results suggest that net primary production was reduced as a whole in our study. While a higher specific leaf area helps intercept more light for photosynthesis, it is also related to a decrease of leaf thickness which in turn would affect leaf ontogenic resistance to apple scab (*Venturia inaequalis* (Cooke) Winter) and to herbivores in non-resistant cultivars (Ballaré and Pierik, 2017). This suggests that further studies should investigate relationships between these shade effects-mediated changes in leaf morphology and pest and diseases symptoms.

Another common and observed shade avoidance trait is stem elongation (Smith and Whitelam, 1997; Valladares and Niinemets, 2008; Ballaré and Pierik, 2017). In our study there was no difference in elongation at the shoot (i.e. internode length) or at the tree (i.e. tree height) scale. However, when looking at two indicators of trunk morphology, slenderness and taper, that integrate tree height (primary growth) and diameter (secondary growth) there were significant differences between light intensities. With light intensity, decreasing apple tree trunk were slender and with a lower taper. These results suggest that rather than the shoot primary growth, it is the whole branch geometry that is affected by light conditions and that those differences appear when secondary growth occurs. One possible reason for these changes in trunk morphology could be linked to carbon assimilation and allocation. Photosynthetic rate is reduced in shade (Evans and Poorter, 2001) leaving less carbohydrates for the different sinks. Carbon allocation has been shown to be modified in shade avoidant species (i.e. root/shoot ratio) (Gommers *et al.*, 2013; Yang and Kim, 2019) and apple tree in shade could prioritize other sinks such as shoots primary growth and roots over secondary growth.

The relative growth rate of bourse shoots was affected by light intensity, but not vegetative shoots. From three to five weeks after full bloom the majority of carbohydrates fixed by rosette (i.e. bourse leaves) and bourse shoot are exported to fruit (Fanwoua *et al.*, 2014). While it has been reported that shade can delay partitioning in apple trees between shoots and fruits (Corelli Grappadelli *et al.*, 1994), the reduction of photosynthetic rate might have exacerbated local competitions for carbohydrates and therefore impeded the growth of bourse shoots. Shade tolerant species are expected to have a higher relative growth rate in shade (Valladares and Niinemets, 2008; Gommers *et al.*, 2013) suggesting that our plant material is not tolerant to shade. Looking at the different traits, it is hard to conclude on an avoidance or tolerance strategy concerning apple tree in shade. In our apple trees some common traits cited in the literature were expressed and well correlated to a reduction in light intensity, but others were not affected (i.e. internode length). A next step would be to study complementary traits (e.g. fruit growth) and validate the hypothesis of a modification of carbon allocation between compartments as well as to explore the genetic variability of these traits.

4.2. Changes in the reproductive strategy

In a fruit tree based agroforestry system, the aim is to produce marketable fruit. In the present study, two essential steps have been studied: floral initiation and fruit set. The latter is the first hurdle that will determine yield during a growing season. While in shade, flowering has been reported to be accelerated (Smith and Whitelam, 1997) or delayed (Lorenzo *et al.*, 2019) depending on the experiment and plant material. Our study partially disagreed with these results. We showed that if the date of budburst was not related to light intensity, the phenology was modified around full bloom. In 2018 and 2019, flower clusters in shade lost their petals prematurely compared to flower clusters in full light. This could lead to a shorter pollination window, less attractiveness to pollinators (Moyroud and Glover, 2017) and could potentially affect negatively fruit set. However, in our study, light intensity did significantly reduce fruit set rate in full light conditions (Figure 2B), suggesting that the petal fall had a limited impact on fruit set rate. Shade even promoted fruit set rate in the apple tree but this result could be a consequence of a lower fruit load at the whole tree scale.

A decrease in light intensity, whether natural or artificial, has a negative impact on floral initiation (Jackson and Palmer, 1977; Smith and Whitelam, 1997). Floral initiation is a complex phenomenon but is known to be correlated to the leaf area of the previous year (Lauri and Trottier, 2004; Buban and Faust, 2011; Belhassine *et al.*, 2019). In our study, a moderate light intensity, i.e. 1124 ± 182 MJ.m^{-2} (reduction of 30% of light intensity) did significantly reduce the leaf area for both years and the number of flower clusters in 2019 but not in 2018. Although our study was carried out only over two years, this suggests that differences between apple trees in different light conditions will be more marked with aging. The lack of light could also extend the period before the apple trees enter a full reproductive stage which, in turn, would influence its flowering pattern (Costes and Guédon, 2012). However, the relation between leaf area of the previous year and the number of flower clusters in the current year (Figure 12) was not modified between full light and moderate light. These two variables were positively correlated in 2019 and 2020. The lack of carbohydrates due to shade slows down the growth and development of the tree but does not seem to affect its fruiting capacity relative to vegetative development. More years of study will be needed before concluding but these results are promising for the future of fruit tree based agroforestry systems built around apple trees. The changeover from an allometric relation between leaf area of the previous year and the number of flower clusters of the current year in 2019 to an isometric relation in 2020 could be a consequence of having fruits on the trees the previous year or linked to the tree’s ontogeny.

Under a certain threshold of light quantity, i.e. 600 ± 138 MJ.m^{-2} (reduction of 65% of light intensity, low light treatment in our study), floral initiation was greatly impeded. Apple trees had a significantly lower leaf area and less flower clusters in 2018 and 2019 in low light. In 2019, there was an average of only 10% of flower clusters on apple trees in low light. Furthermore, there was no correlation between leaf area of the previous year and the number of flower clusters of the current year for trees in low light, suggesting that there are other signals linked to light intensity that prevented floral initiation. However, even a moderate light reduction and a modification in light quality could have a cumulative effect on apple tree development and fruit production (Morandi *et al.*, 2011; Bastías and Corelli-Grappadelli, 2012; Miller *et al.*, 2015; Lopez *et al.*, 2018) which should be investigated on a longer study period.

5. Conclusion

This study showed that apple trees were affected by a gradient of shade and that shade adaptation traits, for both vegetative and reproductive compartments, increased with a decrease in light intensity. While there were architectural and morphological modifications to the expression of these traits, the main concern revolves around floral initiation, the ratio between vegetative and reproductive growth and, to a lesser extent, the phenology of flower clusters. As it stands apple trees that do not receive enough light will not be able to produce fruits in sufficient quantity and, potentially, quality. A recent study on *Arabidopsis thaliana* L. reports that shade avoidance traits become more aggressive in warm environments (Romero-Montepaone *et al.*, 2020), so the shade adaptation traits might be exacerbated in the near future in response to climate change impeding even further their capability to produce fruit. In this regard, apple trees should be planted in between two rows of taller timber trees but not on the row as in our low light treatment. However, apple trees were still young and were not in their full reproductive phase. In agroecological mixed orchards, the time required to reach a productive orchard could be increased, delaying the return of investment for growers. There is still a lot to learn from this experiment as the fruiting patterns of an adult tree might be different to what was observed so far. Looking at regularity of production along the light gradient, will also be critical as irregular bearing has been a main concern for research and growers in the past decades. Pushing further this study by including different genotypes and management practices (e.g. pruning, pollarding walnut trees, coplanting to limit competition for light in the first years, …), will provide valuable insights in fruit tree based agroforestry systems in temperate climate. Finally, even if the production service might be reduced, these agrosystems are interesting for other services such as the generated biodiversity. While they might not all be of the same importance, depending on the actor, it is necessary to consider the system globally and study every aspect of it before concluding on their interest.

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AUTHOR CONTRIBUTIONS

B. Pitchers conducted the investigation and led the writing. P-É Lauri and F.C. Do conceptualized and supervised the study as well as reviewed and edited the paper. C. Pradal led the software development (OpenAlea) and reviewed the paper. L. Dufour managed the resources and reviewed the paper.

DATA AVAILABILITY

The data used in this research was published in the figshare.com data base (https://figshare.com/s/17e79a5c3191a778ea98) and the digital object identifier (10.6084/m9.figshare.13142657).

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CHAPTER 4: *CAN AGROFORESTRY IMPROVE APPLE TREES WATER USE? – AN ESSAY COMBINING ENVIRONMENTAL VARIABLES AND SAP FLOW*

In preparation

Original article

Can agroforestry improve apple trees water use? – An essay combining environmental variables and sap flow

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Abstract

Under soil water restriction, plant growth is impaired by the indirect negative impact on plant carbon balance of stomata closure and hydraulic failure**.** With climate change, water scarcity may be imposed during a significant period of the growing season. Among abiotic stresses, drought has the greatest impact on the morphology, physiology and biochemistry of plants. In this context, it is important to implement strategies to compensate for the negative effects of water stress on growth during drought years and shade has been proposed as a solution to improve tree water status and water use efficiency during drought period. Sap flow density (J_s) of 45 apple trees in their third and fourth year was measured every 30 minutes and used to estimate transpiration (WU) and transpiration per unit of leaf area (E_l). The data was then analysed in regard to light conditions, reference evapotranspiration (ET0) and vapour pressure deficit (D). Our study revealed that the mean daily sap flow density was not affected by environmental conditions but water use and transpiration per unit of leaf area were but there was no effect of shade on water and light use efficiency. WU was negatively impacted by shade, whereas E_l, on the opposite, was positively impacted by shade. Apple trees in moderate and low light were less responsive to an increase in D compared to apple trees in full light suggesting that shade-induced proximity to walnut trees limited the maximum value of J_s in the morning. In 2019 there was an effect of leaf area and the number of ramifications on the cumulated E_l during the summer. Shade did not increase E_l during days with a high evaporative demand and reduced E_l during days with average demand. As leaf area increases and the architecture is more complex (i.e. number of ramifications there was an increase in apple tree E_l during the summer 2019.

Keywords

Agroforestry, apple tree, sap flow, transpiration, architecture, water scarcity

1. Introduction

Among different threats to the sustainability of agriculture, water is becoming a major concern in many parts of the world (Fereres and Soriano, 2006; Levidow *et al.*, 2014). With climate change, years with long drought period are expected to be more frequent in the Mediterranean basin (Pascual *et al.*, 2015) and water scarcity may be imposed during a significant period of the growing season. Among abiotic stresses, drought has the greatest impact on the morphology, physiology and biochemistry of plants (Ma *et al.*, 2010; Jiménez *et al.*, 2013; Bolat *et al.*, 2016). In this context, it is important to implement strategies to compensate for the negative effects of water stress on growth during drought years.

Fruit trees are usually irrigated and their growth and production heavily depend on water availability and irrigation (Mills *et al.*, 1996; Mpelasoka *et al.*, 2001). Nevertheless, in arid and semi-arid regions water stress has become a critical issue in orchards (Naor *et al.*, 2008; Fernández and Cuevas, 2010) and can have positive or negative impact on fruit trees. For example in the apple "Granny Smith" cultivar, a long term water stress decrease the total number of ramification and increase the transition to flowering, short and dead growth units with a higher relative frequency of floral growth units compared with vegetative ones eventually reducing biennial bearing (Yang *et al.*, 2016). Water stress promotes earlier and longer growth cessation, decreases growth resumptions and leaf area, thus modifying tree architecture (Lauri *et al.*, 2016a; Chen *et al.*, 2018). Water stress can also impact fruit development (Berman and Dejong, 1996) through two physiological mechanisms, a reduction in cell turgor (Kramer and Boyer, 1995), and a limitation of carbohydrate availability due to a decrease in photosynthesis rate (Naschitz *et al.*, 2010). A suitable solution to mitigate the negative effects of water stress on apple trees fruit growth should improve the water status of the tree and at least, maintain the tree's ability to reproduce (Lopez *et al.*, 2012).

Shade has been proposed as a solution to improve tree water status and water use efficiency during drought period (Nicolás *et al.*, 2005; Girona *et al.*, 2012). While light interception has been reported to be a primary factor to fruit yield (Palmer *et al.*, 2002) some studies have shown that under a moderate water stress net shading improved yield in apple (Lopez *et al.*, 2018). The benefit of shade is multi-factorial, improving the tree water status, delaying fruit maturity hence giving more time for fruit growth and reducing photo-inhibition. Agroforestry has been reported to increase water use efficiency thanks to a greater distribution of root systems (Gliessman, 1985; Vandermeer *et al.*, 1998; Smith, 2010; Wolz *et al.*, 2018) and has been proposed as an interesting cultural system because it also reduces nutrient and pesticide runoff (Davis *et al.*, 2012), increases biocontrol (Gliessman, 1985), improves soil quality, erosion control and carbon sequestration (Cardinael *et al.*, 2017) and alleviates hazards linked to extreme climatic events (Leakey, 2014). However, to our knowledge, the impact of above and below ground interactions taking place in an agroforestry system on the apple tree water use has yet to be evaluated.

Sap flow probes are routinely used in forest and horticulture as an indicator of water use in response to climatic variables (Poyatos *et al.*, 2016). Among the heat-based methods, the constant thermal dissipation method (CTD, Granier 1985) is the most used system (Poyatos *et al.*, 2016) because of its simplicity (Flo *et al.*, 2019). The transient thermal dissipation (TTD) system is an empirical evolution which retains the simplicity of CTD but reduces the sensitivity to natural temperature gradients and the energy consumption. TTD uses a transient heating of 10 minutes and can be applied to both dual or single needle probes (Do and Rocheteau, 2002; Do *et al.*, 2011).

The objectives of this study were (i) to quantify sap flow density (J_s), water use and the transpiration per unit of leaf area (E_l) of apple trees planted under walnut tree entailing different light conditions in agroforestry, (ii) determine how the light environment influenced J_s and E_l , and (iii) study relations between apple tree architecture at the tree scale and J_s or E_l. Summers in Mediterranean climate are characterised by excessive radiation and temperature (Grappadelli and Lakso, 2007). We hypothesised that apple trees planted near the walnut trees would not have their J_s and E_l impacted over the growing season. However, benefiting from a favourable microclimate and less excessive radiation, we expect apple trees in agroforestry to maintain their stomata open longer than apple trees in full light during summer. Therefore, J_s during specific day, i.e. high vapour pressure deficit (D) should be higher for the apple trees in agroforestry compared to apple trees in full sun. Finally, since different degree of shade impact apple tree architecture (Pitchers *et al.*, 2020), we hypothesised that these differences in architecture could be related to apple tree E_l.

2. Materials and methods

Study site

The study site is located on the "Domaine de Restinclières" (Prades-le-Lez, Hérault, France, 43° 42' 12.168" N, 3° 51' 29.872" E - https://umr-system.cirad.fr/en/the-unit/research-and-training-platform-in-partnership/restinclieres-agroforestery-platform-rap). Apple trees (*Malus domestica* Borkh. cv. 'Dalinette' grafted on Geneva® G202 C.O.V. rootstock) have been planted as an intercrop in March 2016 on a plot with 10 rows of walnut trees (*Juglans nigra* x *Juglans regia* NG23 grown as timber) planted in February 1995 and a legume intercropped (*Onobrychis viciifolia*). Walnut tree rows were planted 13 meters apart and were oriented East West. In 2007, the smallest walnut trees were thinned down in order to promote the bigger ones, leaving a gap of four meters between two walnut trees on the row, i.e. the initial distance between walnut trees within each row, or a multiple of four meters.

Apple trees were planted at various distances from walnut trees, either outside the walnut plot, or between and within walnut rows, so as to create contrasted light conditions.

Apple trees were managed according to organic growing guidelines. Woodchips were spread on apple and walnut-apple rows in order to control weeds. Drip irrigation and organic fertilization were provided to the apple trees following technical advice by an extension service. Apple trees were not pruned to let the natural architecture establish and limit the exogenous factors that could influence the trees response. Apple trees were thinned after full flowering in 2017 and 2018 to prevent the inhibition of floral initiation by gibberellins produced by seeds among other factors (Pratt, 1988; Wilkie *et al.*, 2008), and to limit competition between fruit sinks and promote vegetative growth.

Forty-five apple trees were selected in 2018 depending on both their trunk cross section area and light environment to obtain a gradient for these two variables in each treatment. All the measurements were done in 2018 and 2019.

Air humidity and temperature in weatherproof housing, and global and diffuse solar radiation were recorded every hour at a wheatear station located at 400 meters from our experimental fields with probes (Vaisala HUMICAP® by Campbell Scientific Inc. and BF5 Sunshine Sensor by Delta-T Devices; https://www.campbellsci.com/hmp35a). To check and adjust water irrigation, soil water content was measured at 3 different depths in the soil (20, 40 and 80 cm) under apple trees in the three plantation conditions by water content reflectometer (CS616, Campbell Scientific, Inc. Logan, UT, USA). All the probes were connected to a data logger (CR1000, Campbell Scientific, Inc. Logan, UT, USA).

Light quantification

Hemispherical pictures were taken with a fish-eye lens above each apple tree to evaluate the quantity of photosynthetically active radiation (PAR) received by each of the 45 apple trees. The pictures were taken at dawn to avoid direct sun light, and at two walnut phenological stages, before budburst (minimum shade) and full foliation (maximum shade). Between these two dates a linear regression was used to estimate the quantity of light received each day by the apple trees. Images were processed with the WinSCANOPY™ software (Regent Instruments Inc.). A clustering approach was used to create three groups of apple trees according to the quantity of PAR received during the growing season, full light, moderate light and low light. There were 21 apple trees in the full light group (15 from AC and 6 from AFIR), 15 in the moderate light group (8 from AFIR and 7 from AFR) and 9 in low light (1 from AFIR and 8 from AFR). Over the growing season, the apple trees in each group received respectively 1671 ± 120 MJ of PAR.m^{-2}, 1124 ± 182 MJ of PAR.m^{-2} and 600 ± 138 MJ of PAR.m^{-2}.

Sap flow measurement

Granier-type single probes with a sensitive tip of 2 mm diameter and 8 mm length (UP GmbH, Cottbus, Germany) were used to measure the sap flow of the 45 apple trees. The probes were inserted at the base of the trunk, approximatively 20 cm above the graft point, at budburst in the beginning of march 2018. The general TTD system with single probe was described in Do *et al.* 2011. The heating power was tuned at 0.08 W according to the ratio of 0.1 W per 10 mm of probe dissipation length. Our experiment applied a cycle of 10 min heating and 20 min cooling, which allowed a measurement of flux density every 30 min. The temperature signal was recorded before heating and at intermittent times (1, 2, 3, 4, 5, 10, 30, 60, 120, 300 and 600 seconds) in the heating phase via a data logger (CR1000, Campbell Scientific, Inc. Logan, UT, USA).

To convert the heat signal into a xylem sap flow a calibration was performed in the laboratory. Five branch sections of 20–40 mm in diameter, i.e. covering the range of trunk diameter in the experimental field, and 500mm in length from the same apple tree cultivar were selected. After being cut the surface of active xylem for each branch was measured with a calliper before immersing them in a plastic bag full of water and stored in a cold storage. The allometric relation between active xylem area and mean diameter was established on fifteen branches ($xylem\ area\ (cm^2) = 0.0034 * mean\ diameter^{2.1664}\ (mm),\ r^2 = 0.996$). Before installation, the stem was re-cut under fresh water at both ends to a final stem length of 400 mm. Then, two Granier-type single probes with a sensitive tip of 2mm diameter and 8mm length (UP GmbH, Cottbus, Germany) were inserted into the stem on opposite sides. The spacing was 100mm between probes, and between the probes and the ends of the cut-stems. The stem was maintained in a vertical position and connected to a peristaltic pump (model MS- REGLO, Ismatec SA, Glattbrugg-Zurich, Switzerland) in order to impose different flow rate. The pump was connected to a reservoir containing a KCl solution (20 mM) to prevent the development of microbes and safranine to check the active sap-wood area by staining. The flow rate was controlled by weighing the output with an automatic balance (0.01 g accuracy, Adventurer model, Ohaus, Parsippany, NJ, USA). The pump was managed in order to have a flux density, i.e., the volume of solution going through the stem segment per cross section area and per hour, at 0.5, 1, 3, 5 and 7 $L.dm^{-2}.h^{-1}$ with stabilized steps of 1 h. The thermal index expressed as K_l was then calculated. K_l is based on the ratio between the maximum temperature increase [recorded under zero flow (T_0)], and the increase under the measured flow (T_u) (Isarangkool Na Ayutthaya *et al.*, 2010; Do *et al.*, 2011).

$$K_l = (T_0-T_u) / T_u \text{ (equation 1)}$$

By correlating the thermal index with the J_s, it was possible to determine a second-degree polynomial.

$$J_s = -9.5923K_l^2 + 22.415K_l,\ r^2 = 0.95,\ RMSE = 29.4\% \text{ (equation 2)}$$

SFD was then corrected depending on the temperature of the matrix using a methodology inspired by Flint *et al.* 2002. After testing for the effect of the temperature, a second order polynomial was fitted to a model of prediction of dimensionless slope (S).

$$S = -9e^{-05} * J_s^2 - 5e^{-05} * J_s - 0.0009,\ r^2 = 0.73 \text{ (equation 3)}$$

Equation (3) was then used in an iterative process to correct the heat signal to a matrix temperature of 22°C, (i) an initial estimate of S was obtained by using the SFD obtained with equation (2), (ii) an estimate of the deviation was computed from the equation $d = 22 - S * T_b$ where T_b is the initial temperature measured before the heating and 22 the reference temperature, and (iii) T_u^* was obtained from the equation $T_u^* = T_u + d$. T_u^* was then used in equation (1) to estimate a new SFD. This process was repeated 3 times until the variation of SFD was inferior to 0.1.

Leaf Area

Tree leaf area was estimated in two steps. First, in 2018, two allometric equations between the length on an axis and true leaf area were computed on axes sampled from our experimental plot and depending on light environment (full sun and shaded trees). Two axis categories were considered, vegetative and bourse-shoot, i.e. the shoot branched on the side of the inflorescence, with 30 fully grown axes in each category. True leaf area was measured by scanning all leaves of a shoot and analysing the images with WinFOLIA^{TM}. Second, for both years, 2018 and 2019, inferred leaf area from the allometric relations was computed for each axis category of the described trees and summed up to get total leaf area of each tree.

Ecophysiological variables

While agroforestry is supposed to modify the microclimate, our climate data were recorded in an open field to decipher if agroforestry had an impact on J_s. From this, data air vapour pressure deficit (*D*) was calculated using the equations [1] to [4] from FAO (Allen *et al.*, 1998):

$$D = e_s - e_a \quad [1]$$

$$e_s = \left[\frac{e^0(T)_{max} + e^0(T)_{min}}{2}\right] \quad [2]$$

$$e_a = \frac{e^0(T)_{min}\frac{RH_{max}}{100} + e^0(T)_{max}\frac{RH_{min}}{100}}{2} \quad [3]$$

$$e^0(T) = a\exp\left[\frac{bT}{(T+c)}\right] \quad [4]$$

Where e_s is the saturation vapour pressure (kPa), e_a the actual vapour pressure (kPa), $e^0(T)$ the saturation vapour pressure at the air temperature T (kPa), T the air temperature (°C), RH the air relative humidity) and a, b and c are constants set to 0.6108, 17.27 and 237.3, respectively. Transpiration per unit of leaf area (E_l) was then calculated using ($E_l = (J_s \text{ x } SWA) / LA$ [5]) where J_s is the sap flow density, SWA the sap wood area and LA the leaf area.

The hourly reference evapotranspiration (ET0) was also calculated using the FAO Penman-Monteith equation [5] (Allen *et al.*, 1998):

$$ET0 = \frac{0.408\Delta\,(R_n - G) + \gamma\frac{900}{T_a + 273}\,U_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34U_2)} \quad [5]$$

where *Rn* is the net radiation ($MJ.m^{-2}.day^{-1}$), *G* is the soil heat flux density ($MJ.m^{-2}.day^{-1}$) (soil heat flux was assumed to be negligible), T_a is the mean daily air temperature at a height of 2m (°C), U_2 is the wind speed at a height of 2m ($m.s^{-1}$), e_s is the saturation vapor pressure (kPa), e_a is the actual vapor pressure (kPa), e_s-e_a is the saturation vapor pressure deficit (D, kPa), Δ is the slope vapour pressure curve ($kPa.°C^{-1}$), and γ is the psychrometric constant ($kPa.°C^{-1}$).

Water use efficiency and light use efficiency were calculated as the ratio of dry biomass created over the growing season on transpiration (J_s*SWA) or the quantity of PAR received respectively. Dry biomass was calculated by adding primary and secondary growth. Primary growth biomass was calculated by multiplying the leaf area by the leaf mass per dry area and considering that shoot primary growth was linearly correlated to leaf mass. Secondary dry biomass was estimated by calculating the stem volume of each apple tree knowing every branch length and diameter. The difference between the volume in 2019 and the volume in 2018 was then multiplied by the wood density measured during sap flow probes calibration ($0.75\ g.cm^{-3}$). To estimate the secondary growth in 2018 an allometric relation between tree stem volume and trunk cross section area (TCSA) was established (Volume = 545.85 x TCSA $^{0.9318}$, $r^2 = 0.95$) which allowed to estimate tree stem volume at the beginning of the growing season. The difference between these two volumes was then multiplied by wood density.

As water use is usually well correlated to leaf area, a standardised major axis approach (SMA) for each light conditions was performed (Warton *et al.*, 2012) which allows to compare slope, a possible common slope, and in this case the existence of a shift along the common slope, and the elevation of each regression.

Data analysis

All statistical analysis were done using Rstudio (version 1.2.5001; Allaire, 2012) and with R (R core team, 2020). Packages tydyverse (Wickham *et al.*, 2019) was used for data wrangling, Smatr (Warton *et al.*, 2012) for SMA, car (Fox *et al.*, 2012) to test the significance of the correlations, and Growthcurver (Sprouffske and Wagner, 2016) to compare daily J_s dynamics. All graphs were realised in Microsoft Excel.

3. Results

Seasonal courses of climate, D, ET0 and water use

Mediterranean climate is characterized by mild wet winters and warm to hot, dry summers (Lionello *et al.*, 2006). The climate diagrams for 2018 and 2019 (Figure 14A) confirmed this trend and showcased that Mediterranean climate is highly variable. While 2018 was characterized by exceptionally high precipitation in spring and a hot and dry summer, 2019 was dryer and temperature peaked at 47°C on the 28th of June. These dry summers impact soil water content down to 80 cm depth going from 0.5 m^3 of water per m^3 of soil to 0.4 at the end of the summer. Drip irrigation while allowing to maintain the apple trees in a comfort zone was not enough to compensate for the evapotranspiration and the base line followed the same trend that at 80 cm depth.

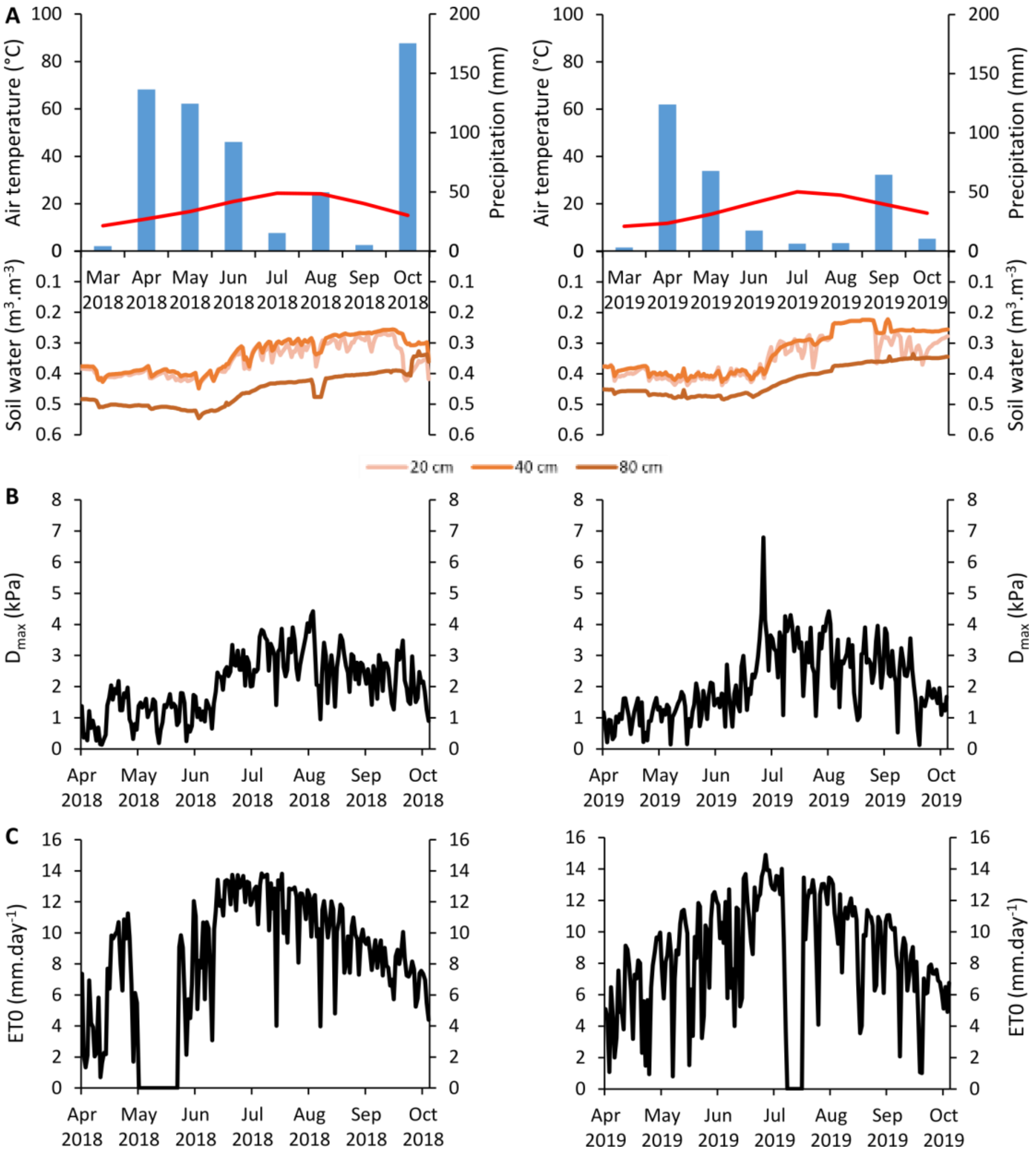


Figure 14: Climate variables in the experiment from April to October 2018 and 2019. A. Average daily temperature (°C; red line) and cumulated precipitation per month (mm; blue bar plots). At the bottom soil water content (m3.m-3) is represented at three different depths (light brown: 20 cm, medium brown: 40cm, dark brown: 80 cm). B. Daily maximum water pressure deficit. C. Daily reference evapotranspiration.

These summers reflect in the daily maximum value of vapour pressure deficit (D_{max}) over the apple trees growing season (Figure 14B). For both years, between March and June, D_{max} varies between 1 kPa and 2 kPa before increasing between 3 kPa and 4 kPa until the end of September. During the summer, D_{max} can occasionally reach values above 4 or even 6.8 on the 28th of June 2019. Evapotranspiration (ET0; Figure 14B) was calculated during the growing season of the apple trees. In May 2018 and during a week in July 2019, due to a technical problem, there was no data collected from the pyranometer. ET0 increased gradually during the growing season from 4 mm a day to 13 mm in July before decreasing back to 6 mm at the beginning of October (Figure 14C).

Table 3: Summary of Malus domestica Borkh cv. 'Dalinette' structural and functional variables on which sap flow measurement were performed for two years. Values are mean±SE. Kruskall Wallis rank test followed by Tukey's multiple comparison test were performed. In a same line, different alphabetical letters denote significantly different means at $P < 0.05$.

	Full light	**Moderate light**	**Low light**
Number of apple trees	21	15	9
PAR received (MJ/m^2)	1671 ± 120 ^{a}	1124 ± 182 ^{b}	600 ± 138 ^{c}
Structural variables			
Aboveground biomass increase (kg of dry matter)			
2018	2.07 ± 0.83 ^{a}	1.38 ± 0.29 ^{b}	1.00 ± 0.16 ^{c}
2019	3.09 ± 1.59 ^{a}	1.82 ± 0.54 ^{ab}	1.26 ± 0.35 ^{b}
Sap wood area (cm^2)			
2018	4.14 ± 2.02 ^{a}	2.56 ± 0.62 ^{b}	1.76 ± 0.36 ^{c}
2019	6.34 ± 3.87 ^{a}	3.57 ± 1.24 ^{ab}	2.41 ± 0.70 ^{b}
Specific leaf area ($cm^2.g^{-1}$)	149.4 ± 10.5 ^{b}	164.5 ± 12.1 ^{a}	170.3 ± 10.6 ^{a}
Leaf area (m^2)			
2018	2.16 ± 0.68 ^{a}	1.67 ± 0.39 ^{ab}	1.23 ± 0.18 ^{b}
2019	3.53 ± 1.31 ^{a}	2.44 ± 0.60 ^{b}	1.86 ± 0.26 ^{c}
Huber value ($m^2.m^{-2}$)			
2018	1.6e-04 ± 5.1e-05 ^{a}	1.4e-04 ± 2.4e-05 ^{a}	1.3e-04 ± 2.4e-05 ^{a}
2019	2.1e-04 ± 4e-05 ^{a}	1.8e-04 ± 2.3e-05 ^{ab}	1.5e-04 ± 2.4e-05 ^{b}
Functional variables			
J_s ($L.dm^{-2}.day^{-1}$)			
2018	36.0 ± 15.5 ^{a}	37.0 ± 14.4 ^{a}	37.0 ± 14.3 ^{a}
2019	38.2 ± 17.7 ^{a}	39.3 ± 16.6 ^{a}	39.4 ± 16.2 ^{a}
Water Use ($L.day^{-1}$)			
2018	1.16 ± 0.79 ^{a}	0.79 ± 0.40 ^{b}	0.55 ± 0.23 ^{c}
2019	2.46 ± 1.67 ^{a}	1.22 ± 0.63 ^{b}	0.78 ± 0.31 ^{c}
E_l ($mm.day^{-1}$)			
2018	0.60 ± 0.32 ^{a}	0.53 ± 0.25 ^{b}	0.50 ± 0.25 ^{c}
2019	0.69 ± 0.38 ^{a}	0.59 ± 0.32 ^{b}	0.47 ± 0.28 ^{c}
Water Use Efficiency ($g.L^{-1}$)			

2018	7.7 ± 0.3^{a}	9.0 ± 2.0^{a}	9.2 ± 1.2^{a}
2019	8.6 ± 2.0^{a}	9.0 ± 1.6^{a}	10.5 ± 2.3^{a}
Light Use Efficiency ($g.MJ^{-1}$)			
2018	1.23 ± 0.46^{b}	1.23 ± 0.18^{b}	1.72 ± 0.37^{a}
2019	1.82 ± 0.87^{a}	1.63 ± 0.38^{a}	2.07 ± 0.44^{a}

Sap flow density (J_s) did not seem to follow the trend of D and ET0 (Figure 15A) and was not significantly different between light treatments in 2018 and 2019 (Table 3). In 2018, J_s was stable over the growing season, stabilizing around 40 $L.dm^{-2}.day^{-1}$ from April to the middle of June before slightly increasing to an average closer to 50 $L.dm^{-2}.day^{-1}$ until the end of August and slowly decreasing to an average of 30 $L.dm^{-2}.day^{-1}$ at the beginning of October. Furthermore, there was graphically no clear differences between the apple trees planted in the different light conditions except during the second half of September where cumulated J_s for the apple trees in full light was higher compared to apple trees in moderate and low light conditions (Figure 15B). In 2019, J_s was low in April, around 30 $L.dm^{-2}.day^{-1}$, before increasing to an average of 50 $L.dm^{-2}.day^{-1}$ in May and decreasing slowly to 30 $L.dm^{-2}.day^{-1}$ by the beginning of October. When looking at cumulated J_s in 2019 (Figure 15B), apple trees planted in moderate and low light had a higher sap flow density compared to apple trees planted in full light during May but this difference disappeared in September when J_s was higher for apple trees in full light.

Water use (J_s x SWA) was significantly different between apple trees planted in the different light treatment in 2018 and 2019 (Table 3). Apple trees planted in full light had higher transpiration than trees in moderate light which in turn had higher transpiration than apple trees in low light (Figure 15C). The significant difference in water use was mainly a consequence of a significant difference in SWA. Indeed, SWA was significantly different between all light treatments in 2018 with apple trees in full light having a higher SWA compared to moderate light whom in turn had a higher SWA than apple trees in low light (Table 3). In 2019, apple trees in full light still had a significantly higher SWA compared to apple trees in low light and apple trees in moderate light were not significantly different. In 2018, the differences in water use were really marked during the months of July and September. From April to the first half of June water use was at an average of 0.7 $L.day^{-1}$ for all apple trees. From this time apple trees in full light had an increase in water use to an average of 1.3 $L.day^{-1}$ to the end of august when water use increased to 2 $L.day^{-1}$ in September. Apple trees in moderate light had an increase in average water use to 1 $L.day^{-1}$ until the end of August which decreased to 0.7 $L.day^{-1}$ in September. Apple trees in low light had an average water use that stayed constant over the growing season. In 2019, the difference in water use were important since May to the end of the growing season. Apple trees in full light had an average water use at 1 L.day^{-1} in April, 2.5 L.day^{-1} between May and beginning of July, 4 L.day^{-1} in august and 3 L.day^{-1} in September. Apple trees in moderate light had an average water use at 0.5 L.day^{-1} in April and 1.5 L.day^{-1} and apple trees in low light at 0.5 L.day^{-1} in April and 1 L.day^{-1} the rest of the season. During august water use for apple trees in moderate and low light was similar.

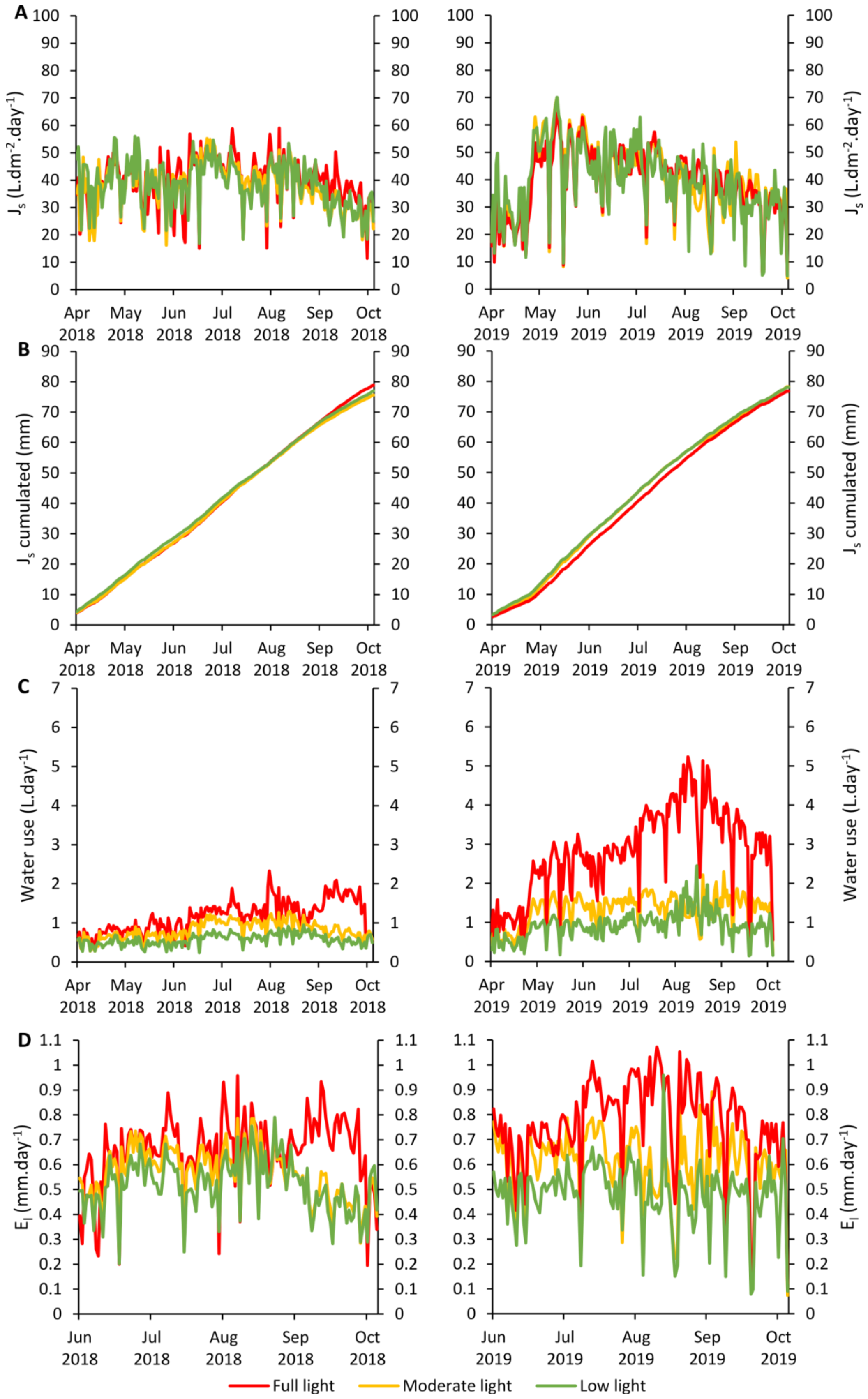


Figure 15: A. Mean daily variation of sap flow density (J_s) for apple trees in the different light conditions (i.e. full light, moderate light and low light) during the two growing seasons. B. Cumulated mean J_s over the growing season for apple trees in the different light conditions. C. Mean daily water use for apple trees in different light conditions for each growing season. D. Mean transpiration per unit of leaf area for apple trees in different light conditions and each growing season from June to October when leaf area is maximum.

Transpiration per unit of leaf area (E_l) was graphically different for apple trees in the different light conditions with apple trees in full light having higher E_l than apple trees in moderate light which in turn had higher E_l than apple trees in low light (Figure 15D). That was confirmed when comparing the mean daily value of E_l of apple trees in the different treatment over the growing season (Table 3). In 2018 and 2019 apple trees in full light had the highest daily mean value of E_l compared to apple trees in moderate and low light and apple trees in moderate light had a significantly higher E_l than apple trees in low light. In 2018, E_l was quite similar between the 3 treatments (0.6 $mm.day^{-1}$) from June to the end of August with short periods where differences were more marked, i.e. one week in July and a couple of days in August. In September however, apple trees in full light had an E_l at 0.7 $mm.day^{-1}$ while apple trees in moderate and low light had and E_l at 0.45 $mm.day^{-1}$. In 2019, differences between the different light treatments were marked from budburst to fall. During the month of June, the average E_l of apple trees in full light was around 0.7 $mm.day^{-1}$ increasing to 0.9 $mm.day^{-1}$ during July and August before dropping to 0.8 $mm.day^{-1}$ in September. For apple trees in moderate light E_l was at an average of 0.7 $mm.day^{-1}$ from the beginning June until the first half of July and dropped to 0.6 $mm.day^{-1}$ until the end of August before slowly increasing to 0.65 $mm.day^{-1}$ in September. Apple trees in low light had an E_l which fluctuated around the average of 0.5 $mm.day^{-1}$ over the growing season.

Influence of environmental variables on apple trees

Aboveground biomass dry matter (not considering fruit in 2019) was significantly different for apple trees in each light condition in 2018, with those in full light establishing more biomass than those moderate light which in turn created more vegetative biomass than apple trees in low light (Table 3). In 2019, apple trees vegetative aboveground dry biomass in moderate light was not significantly different from apple trees in full and low light but apples tress in full light did make significantly drier biomass than apple trees in low light.

Specific leaf area and leaf area was also significantly affected by light conditions. SLA was significantly lower in full light compared to trees in low and moderate light conditions. In 2018, apple trees in low light condition had a significantly lower leaf area than apple trees in full light and apple tree leaf area in moderate light was not significantly different from trees in the other two conditions. Huber value (SWA / leaf area) was negatively affected by the light conditions in 2019 but not in 2018 being significantly higher for apple trees in full light compared to apple trees in low light, with intermediate values for apple trees in moderate light.

Water use efficiency (WUE) in 2018 and 2019 and light use efficiency (LUE) in 2019 over the growing season was not improved by shade in our study as there was so significant differences between apple trees in the three light treatments. However, in 2018, LUE was significantly higher for apple trees in low light compared to apple trees in full and moderate light (Table 3).

Variations of J_s, D and ET0 in periods with high differences between light conditions

From Figure 15 we knew that the differences occurred during the first week of august and September in 2018 for which we chose to focus on the second week where the differences were more important. In 2019, we chose to focus on the last days of June when the air temperature reached 47°C and the first days of August since after the 8th of August some sensors stopped functioning reducing the number of replicates. During these periods, there was a global trend where J_s increased when D and ET0 increased (Figure 16). While for apple trees in full and moderate light there did not seem to be a threshold and J_s kept increasing with D and ET0, for apple trees in low light there seem to be a threshold around 5 $L.dm^{-2}.h^{-1}$. During the first week of august 2018 similar patterns were observed for J_s in the different treatment (Figure 16A) except that apple trees in full light tend to have a higher J_s a few hours after dawn which is then down regulated to a similar pattern as for apple trees in moderate and low light. During the second week of September 2018 J_s for apple trees in full light was almost systematically always higher compared to apple trees in moderate and low light (Figure 16B). Furthermore, J_s for apple trees in full light was relatively stable from one day to the other and still linked to D and ET0. However, for apple trees in moderate light conditions there was an unexpected drop in J_s on the 10th of September followed two days later by apple trees in low light. For these apple trees there was no longer a connection between D and ET0 and J_s. During the first week of July 2019 the same dynamics of J_s were observed between the apple trees in different light conditions except for apple trees in moderate light having a higher J_s than apple trees in low light (Figure 16C). However, J_s in august 2019 was like the pattern from 2018 with apple trees in moderate and low light having similar dynamics and usually inferior to J_s for apple trees in full light (Figure 16D). In conclusion, looking at J_s dynamics according to D and ET_0 dynamics during the periods where the transpiration is the highest showed that apple trees in full light had higher J_s than apple trees in moderate and low light even if there was no statistical differences in J_s when considering the whole growing season. J_s also seemed correlated to D and ET0.

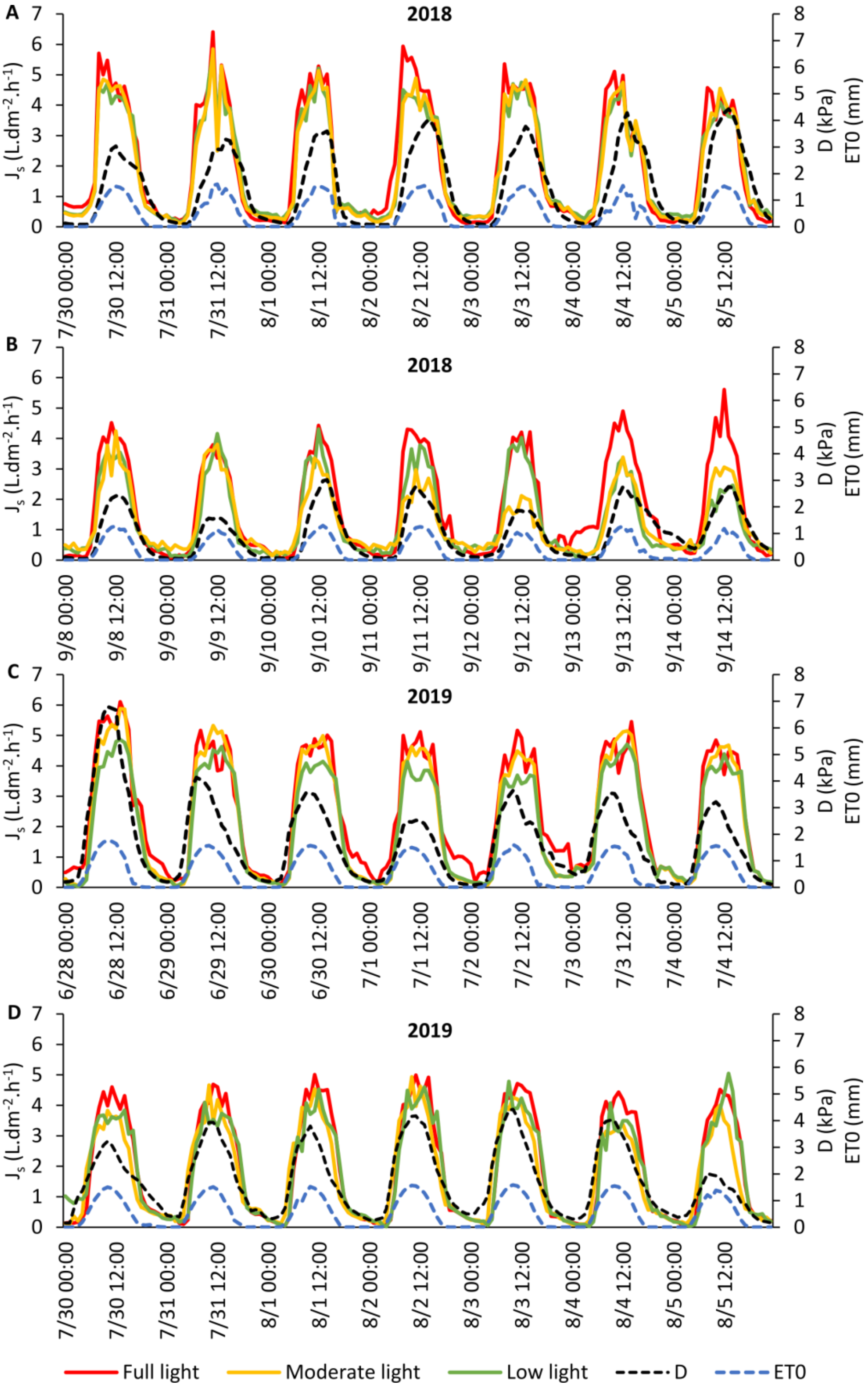
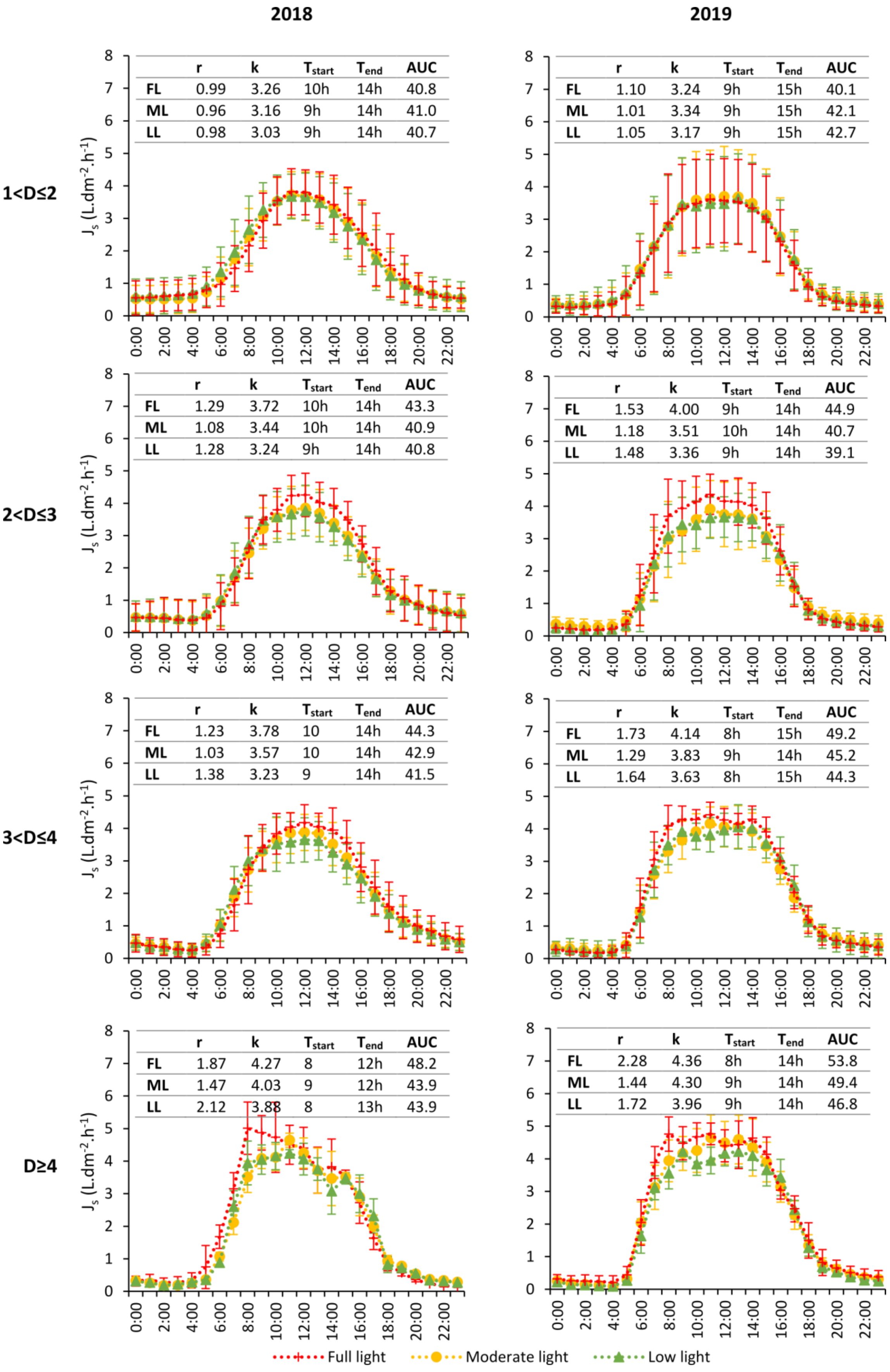


Figure 16: Mean daily dynamics of J_s for apple trees in each light condition during weeks where there was visible differences. The dashed lines represent daily dynamics of D and ET_0.



2018, 1<D≤2

	r	k	T_{start}	T_{end}	AUC
FL	0.99	3.26	10h	14h	40.8
ML	0.96	3.16	9h	14h	41.0
LL	0.98	3.03	9h	14h	40.7

2019, 1<D≤2

	r	k	T_{start}	T_{end}	AUC
FL	1.10	3.24	9h	15h	40.1
ML	1.01	3.34	9h	15h	42.1
LL	1.05	3.17	9h	15h	42.7

2018, 2<D≤3

	r	k	T_{start}	T_{end}	AUC
FL	1.29	3.72	10h	14h	43.3
ML	1.08	3.44	10h	14h	40.9
LL	1.28	3.24	9h	14h	40.8

2019, 2<D≤3

	r	k	T_{start}	T_{end}	AUC
FL	1.53	4.00	9h	14h	44.9
ML	1.18	3.51	10h	14h	40.7
LL	1.48	3.36	9h	14h	39.1

2018, 3<D≤4

	r	k	T_{start}	T_{end}	AUC
FL	1.23	3.78	10	14h	44.3
ML	1.03	3.57	10	14h	42.9
LL	1.38	3.23	9	14h	41.5

2019, 3<D≤4

	r	k	T_{start}	T_{end}	AUC
FL	1.73	4.14	8h	15h	49.2
ML	1.29	3.83	9h	14h	45.2
LL	1.64	3.63	8h	15h	44.3

2018, D≥4

	r	k	T_{start}	T_{end}	AUC
FL	1.87	4.27	8	12h	48.2
ML	1.47	4.03	9	12h	43.9
LL	2.12	3.88	8	13h	43.9

2019, D≥4

	r	k	T_{start}	T_{end}	AUC
FL	2.28	4.36	8h	14h	53.8
ML	1.44	4.30	9h	14h	49.4
LL	1.72	3.96	9h	14h	46.8

Figure 17: Mean daily J_s dynamics of the apple trees in the different light conditions (FL: full light, ML: moderate light and LL: low light) for different rang of D and for each year. For each graph, the slope of the exponential phase (r), the value of the maximum (k), the time at which 90% of the maximum J_s was reached

(T_{start}) and ended (T_{end}) and the aera under curve (AUC) is indicated in the table above. Within each graph, all statistical differences between light conditions were significant at $P < 0.001$.

We then looked at the average daily dynamics in J_s for different range of D in 2018 and 2019 (Figure 17). In 2018, for a value of D between 1 and 2 (kPa) there was no clear difference between the light treatments, slope (r), maximum (k), and area under curve (AUC) were similar. Apple trees in full light reached 90% of the maximum value (k) one hour after apple trees in moderate and low light while having a higher slope suggesting that there started transpiring later or that their period of latency before the exponential phase was longer. For mean J_s dynamics in 2018 when D was between 2 and 3 (kPa) apple trees in full light had a higher k, and AUC compared to apple trees in low and moderate light, r was similar with apple trees in low light. Apple trees in moderate and low light had a similar AUC but in low light r was higher and k lower. However, apple trees in low light reached 90% of k maximum value 1 hour earlier than apple trees in moderate and full light explaining why AUC between moderate and low light was similar. When D was in a range between 3 and 4 (kPa), the same trend was observed with apple trees in full light having a higher k and AUC compared to apple trees in the other treatments. Slope (r) was highest for apple trees in low light and k the lowest once again allowing them to reach 90% of k maximum value 1 hour before apple trees in full and moderate light. However, this time around that was not enough to compensate for the lower value of k and apple trees in low light had the lowest AUC. When D was higher than 4 (kPa) apple trees in full light had the highest k and AUC and r in between low light and moderate light treatment. Apple trees in low light had the highest r once again and the lowest k and the same AUC than apple trees in moderate light who had a lower r but a higher k. Interestingly, when D was above 4 kPa 90% of the maximum value of k was reached and ended earlier than before. Apple trees in low light and full light reached this threshold at 8am instead of 9am before and J_s was down regulated at noon instead of 2pm. In 2019, when D was between 1 and 2 (kPa) r and the time at 90% of k maximum value was similar for each treatment. However, there was a difference in k, apple trees in moderate light had the highest J_s value and apple trees in low light the lowest. However, when looking at the AUC, apple trees in low light and moderate light were close and higher than apple trees in full light. When D was between 2 and 3 (kPa) in 2019, k and AUC was the highest for apple trees in full light. They reached 90% of the maximum value of k at 9am like apple trees in low light. Apple trees in low light had the lowest value of k and AUC and apple trees in moderate light had the shortest period of time at 90% of k resulting in a AUC close to apple trees in low light. When D was between 3 and 4 (kPa) in 2019, apple trees in full light had the highest value for all curve properties (i.e. r, k, time at 90% of k and AUC). Apple trees in low light had the lowest value of k and AUC but a r higher than apple trees in moderate light. When D was higher than 4 apple trees in full light had the highest value for all curve properties and apple trees in low light the lowest value except for the slope of the increase (r). Furthermore, apple trees in full light reached 90% of the maximum value ok k 1 hour before the apple trees in moderate and low light. Apple trees in moderate light had a value of k close to apple trees in full light and the lowest value of r. In conclusion to this graph apple trees in full light daily dynamic of Js is more sensitive to D than apple trees in moderate and low light. They have a higher maximum value when D is above 2 kPa and are able to maintain it the longest in 2019, in 2018 apple trees in low light were maintaining J_s the longest. Apple trees in moderate and low light were less sensitive to D and had similar AUC but different dynamics. Usually, apple trees in low light had a higher slope during the exponential increase of J_s than apple trees in moderate light but a lower maximum value. Furthermore, apple trees in low light were able to maintain a high value of J_s longer than apple trees in moderate light but were less sensitive to D.

We then explored how J_s and E_l reacted to D_{max} and ET0 (Figure 18). There was a good correlation between J_s, E_l and D_{max}, ET0 in 2018 and 2019 and the best fit was a power curve. D_{max} explained between 50 and 60% of the variability of J_s and E_l in 2018 and 2019 over the growing season. For J_s there was no difference in the fitting curves between light treatments. For E_l there was graphically differences in the fitting curves with a shift in elevation. Apple trees in full light had a higher E_l than apple trees in moderate and low light. This difference in elevation increased when D_{max} increased meaning that the higher the values of D_{max} the higher the differences. Apple trees in moderate light had also a higher E_l compared to apple trees in low light. Furthermore, the shift in elevation increased between 2018 and 2019. ET0 explained between 50 to 80% of the variability of J_s and E_l in 2018 and 2019. The same patterns were observed for both ET0 and D_{max}. J_s was positively correlated to ET0 and there was no difference between the different light treatment. E_l was positively correlated to ET0 as well but there was difference between light treatments. Like for D_{max} there was a shift in elevation between the curves of the different light treatment. Apple trees in full light had a higher E_l for the same value of ET0.

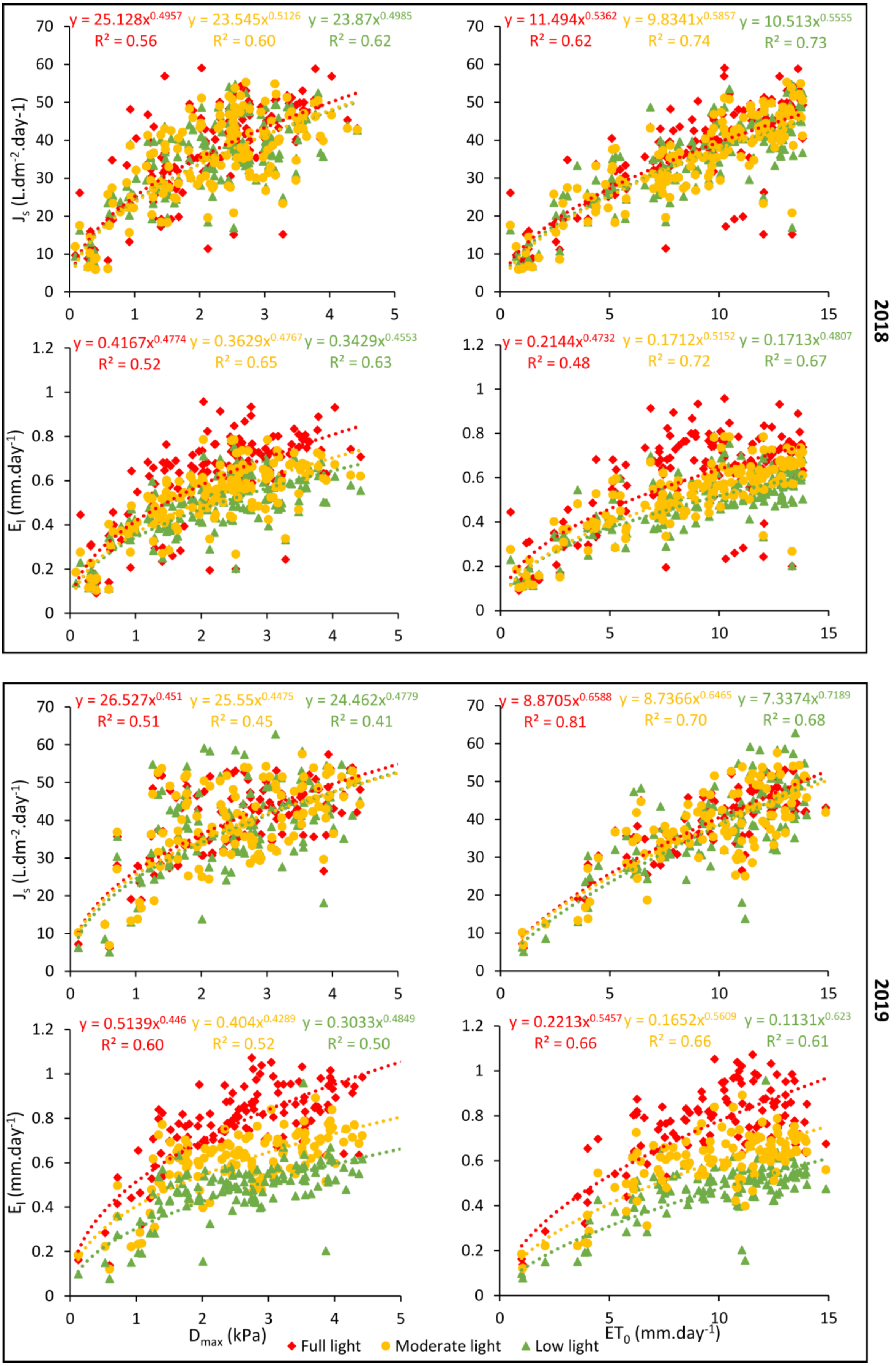
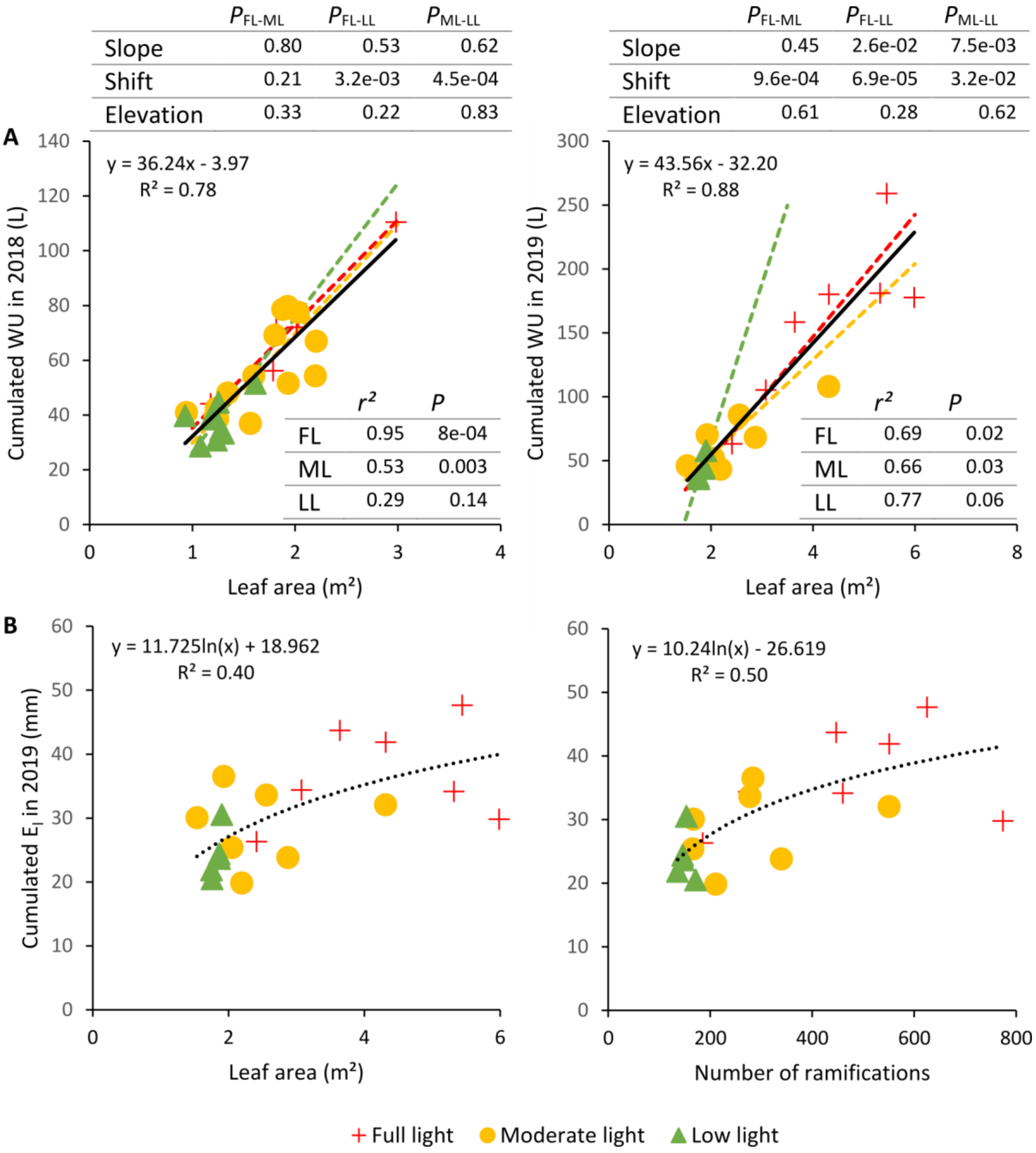


Figure 18: Correlations between daily J_s and E_l and environmental variables (D_{max} and ET_0) for apple trees in the FL: full light, ML: moderate light and LL: low light conditions, in 2018 and 2019.

Finally, knowing that tree architecture was modified by the radiative environment, we looked at the relation between WU and leaf area in 2018 and 2019 (Figure 19A). When pooling all the apple trees together, there was a strong correlation between those two variables. 78% and 88 % of the variability in WU during the summer was explained by leaf area in 2018 and 2019 respectively. When performing this correlation for each treatment, the relation was still very significant for apple trees in full light and moderate light but no longer for apple trees in low light. Leaf area explained 95% and 69% of the WU during the summer for apple trees in full light in 2018 and 2019 respectively, and 53% and 66% for apple trees in moderate light. There were no significant differences when comparing the curve for each treatment except for a shift along the common slope. Apple trees in full light had a higher leaf area than apple trees in moderate light and, therefore, a higher WU during the summer. However, although leaf area and the number of ramifications explained 40% and 50%, respectively, of the variability of cumulated E_l in 2019, these two variables explained less than 1% of the variability of cumulated E_l in 2018.



	P_{FL-ML}	P_{FL-LL}	P_{ML-LL}
Slope	0.80	0.53	0.62
Shift	0.21	3.2e-03	4.5e-04
Elevation	0.33	0.22	0.83

	P_{FL-ML}	P_{FL-LL}	P_{ML-LL}
Slope	0.45	2.6e-02	7.5e-03
Shift	9.6e-04	6.9e-05	3.2e-02
Elevation	0.61	0.28	0.62

	r^2	P
FL	0.95	8e-04
ML	0.53	0.003
LL	0.29	0.14

	r^2	P
FL	0.69	0.02
ML	0.66	0.03
LL	0.77	0.06

Figure 19: Standardized major axis correlation between cumulated WU and leaf area (A) and the number of ramifications (B) during the month of July and August 2018 and 2019, for all apple trees (black line; OLS regression) and for apple trees in different light conditions, FL: full light (red), ML: moderate light (orange) and LL: low light (green) (dashed lines). Two by two comparison results of different parameters (slope, shift along the common slope and elevation) are reported in the table above the figure.

We then correlated E_l to leaf area or the number of ramifications during the summer 2019, when thermal stress and differences were the most important (Figure 19B). A logarithmic relation was the best fit for these correlations and explained 40% and 50% of the variability of cumulated E_l respectively. In 2018, these relations were not significant and explained less than 1% of the variability of cumulated E_l.

4. Discussion

Some studies have emphasized on the interest of using shade or hails nets during critical period to mitigate extreme climatic events (Nicolás *et al.*, 2005; Girona *et al.*, 2012; Lopez *et al.*, 2018). Our study revealed that the mean daily sap flow density was not affected by environmental conditions, but water use and transpiration per unit of leaf area were. WU and E_l were negatively impacted by shade. J_s dynamics were affected by light conditions especially during summer. Apple trees in moderate and low light were less responsive to an increase in D_{max} compared to apple trees in full light. These results suggest that shade-induced proximity to walnut trees limited the maximum value of J_s in the morning. Finally, in 2019 there was an effect of leaf area and the number of ramifications on the cumulated E_l during the summer.

Differences in morphology and architecture are related to differences in water use

Looking at data over the 2018 and 2019 growing seasons there was no effect of light conditions on apple trees J_s. However, apple trees architecture and development were affected by light conditions (Pitchers *et al.*, 2021). Above ground dry biomass was affected by the light conditions in 2018 and 2019 which probably is a consequence of a reduction in photosynthetic rate (Charbonnier *et al.*, 2017) due to light quantity being not enough to saturate the leaf photoreceptors. In 2018, the above ground dry biomass was significantly different among light condition but in 2019 apple trees in moderate light were not significantly different from apple trees in full and low light which were different from each other. This result could indicate that these differences in above ground dry biomass could decrease with the apple trees ontogeny and their architecture getting more complex. However, it is probably an artefact due to the presence of fruits in 2019 that were completely removed in 2018. Indeed, in 2019 5 fruits per cm^2 of trunk cross section area were left when possible and apple trees in full light almost always reached the objective while fruit in moderate light was very heterogeneous as apple trees in low light. In our study, fruits were not taken into account in the aboveground dry biomass while they are a strong sink for carbohydrate resulting in under estimating the above ground dry biomass for all apple trees (Palmer *et al.*, 2002). However, apple trees in full light were more impacted than apple trees in moderate and low light having more fruits. The possible lack of carbohydrate because of shade also affected primary and secondary growth with SWA and LA being significantly lower for apple trees in shade. This resulted in a significantly lower Huber value in 2019 for the apple trees in low light, which would result in a decreased capacity of stem to transport water to leaf (Carter and White, 2009). Transpiration per unit of leaf area confirmed this tendency, in full light apple trees had a higher E_l which could be related to an increase in water transport to leaf. The differences in SWA also led to differences in water use. While this could be interesting in case of water scarcity to save water, orchards are usually irrigated to maintain apple trees in optimal conditions. Furthermore, there was no difference in water use efficiency in 2018 and 2019, so there was a proportional decrease in above ground dry biomass with the decrease in transpiration. In this regard, shade can be used as a mean to face water scarcity by limiting transpiration and could be used as a tool to help growers face particular climatic hazards (Lopez *et al.*, 2018). Interestingly enough light use efficiency was significantly higher for apple trees in low light in 2018 validating results observed on alfalfa (Querné *et al.*, 2017). However, in 2019 there was no differences in LUE suggesting that LUE differences might disappear with apple trees ontogeny or that the balance between above ground and below ground dry biomass can shift between years.

J_s *daily dynamics adapts to a fluctuating environment*

Daily sap flow density over the growing season was similar for all light conditions in 2018 and 2019 (Table 3). However, differences were observed when looking at daily sap flow dynamics specifically during summer when D and ET0 were high. In both years, when D and ET0 increased, there was an increase in the maximum value of J_s that changed according to the light quantity. In a low light environment apple trees J_s maximum was lower compared to apple trees in moderate and full light conditions suggesting that they were less sensitive to the light environmental or controlling they transpiration earlier. Therefore, agroforestry or shade could act as a buffer facing extreme climatic events.

We also observed that for values of D_{max} above 4, J_s in the morning peaked for apple trees in full light before being down regulated to the same level as apple trees in moderate and low light. While we do not have a dynamic of photosynthetic rate that would help us conclude on the interest of this peak in J_s, this water transpired in the morning could represent a net loss in relative extractible water especially during summer when temperature is high and facing water scarcity. Lower J_s could also be interesting in an orchard facing water stress by limiting transpiration in the morning and maintaining relative extractible soil water high enough.

E_l *sensitive to environmental variables and shade*

Water use was impacted by light conditions but mainly as a result of differences in SWA rather than shade. However, correlation between leaf area and WU during summers were no longer significant. While this observation is probably a consequence of the low number of trees in the low light conditions (9 apple trees in 2018 and 5 in 2019) it could also indicate a change of well-established relations like the number of flower clusters to leaf area (Pitchers *et al.*, 2021). Apple trees in low light could also experience shade at different moment during the day inducing different J_s patterns (Miller *et al.*, 2015) that could lead to difference in WU over a long period.

E_l was impacted by shade and environmental variables. There is power relation between E_l and D_{max} or ET_0. The shape of the curve is similar for all light treatments and E_l increases with increasing value of D_{max} and ET_0. In the field, we were not able to evaluate the breakpoint after which we would observe a drop in E_l and if this break point would be different according to the light conditions. While the curve between shade treatments were similar as stated before there was a difference in the slopes. Apple trees in full light were more responsive to environmental variables and these differences with apple trees in other light environment increased with increasing values of D_{max} and ET_0. This suggests that, as opposed to what was hypothesised, shade did not increase E_l during days with a high evaporative demand but that it even impeded E_l further than during days with average temperatures. While there can be several reasons behind this behaviour, we observed that cumulated E_l during the summer 2019 was well correlated to leaf area and the number of ramifications of apple trees. As leaf area increases and the architecture is more complex, apple trees are more efficient at transpiring water per unit of leaf area. However, Lopez *et al.* 2018 have shown that shade can be beneficial in case of low water stress during summer but not when apple trees are well watered which was the case in our experiment. In this regard, agroforestry could have a positive effect on apple trees functioning in case of water stress. Apple is generally considered an isohydric (Jones, 2013), with strong stomatal control as opposed to anisohydric species with a weaker stomatal control upon water scarcity (Tardieu and Simonneau, 1998). Thus, it would be necessary to create different gradient of water stress to fathom the potential benefit of agroforestry regarding water scarcity issue and fathom how shade and water scarcity interacts in this context. Especially when considering that both genetic variability and phenotypic plasticity are involved in the range of iso-anisohydry morphological responses of apple trees to contrasted drought conditions (Lauri *et al.*, 2016b).

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CHAPTER 5: *DISCUSSION*

1. Overview and main results of the thesis

1.1. *Light management is essential in AT-AFS*

To optimize Agroforestry Systems (AFS) in general, understanding interactions between plants to maximize positive interactions and minimize negative interactions is essential (Gliessman, 1985). Our study showed that when working in complex agroforestry systems using a continuous environmental indicator that helps characterize the interactions for each tree can complement analysis using only treatments as the independent factor because of the heterogeneity within a treatment. Distance-dependent crowding models like Neighbourhood Crowding Index (NCI) has proven to be relevant to model tree architectural features but less than considering the limiting factor (i.e. light in this study) and quantifying it. NCI considers that there is no specific limiting factor but that several resources will be simultaneously limiting apple trees growth. As light was hypothesised to be the limiting factor in this study, we used two methodologies to evaluate the Photosynthetically Active Radiation (PAR). The first one estimated the PAR received during the growing season using hemispherical photographs (PAR_{HP}) and the second one the PAR received during full foliation, i.e. when shade was maximum, using a terrestrial scanner (PAR_{TLS}). On the other hand,

All three indicators, NCI and the two PAR methodologies, were promising at explaining the variability of different architectural and morphological traits but the PAR estimated from the terrestrial scanner stood out. According to our results it appears that considering light was always better at explaining the variability of the different measured traits than NCI in 2018 and 2019 confirming that, in the specific context of our study, aboveground interactions were prevalent likely because of irrigation and fertilization that could minimize underground interactions. In other words, the management of the experimental plot participated in shifting the balance between above and belowground competition compared to a ‘natural’ ecosystem. We also showed that the relation between the number of flower clusters and the quantity of light might not be as straightforward compared to TCSA or Leaf area and that a reduction in PAR increases the variability of the number of flower clusters. These results suggested that beyond the effects on vegetative growth, light also affect the relationships between vegetative and reproductive growth.

We showed that the methodologies used to quantify the limiting factor, light here, should be considered beforehand and chosen adequately depending on the study aims and resources. Using terrestrial LIDAR which permits to compute both tree canopy volume and intercepted

PAR is more accurate and precise than using hemispherical photographs but comes at a higher cost and longer analysis. The evaluation of PAR with the terrestrial scanner takes into account the volume of the canopy of apple tree and the light from all directions while the hemispherical pictures returns the quantity of PAR normalized on a square metre (Vincent *et al.*, 2017). Consequently, for PAR_{HP} all apple trees in the control receive approximately the same quantity of light while for PAR_{TLS} apple trees absorbed different amounts of light. In this regard, the radiative balance estimated with a terrestrial scanner is more precise and encompasses more information when used with woody perennial plants likely related to the fact that it takes more into account light coming from the various directions which is known to have effects of plant growth and functioning (Zhang *et al.*, 2020). If working with hemispherical photographs, further analyses should estimate the area projected on the ground of each apple trees to improve environmental indicator.

In the present study, the apple tree based agroforestry system is still young and the balance between above and belowground interactions might still shift especially when considering that drip irrigation will favour the presence of the root systems in the same area as neighbouring trees. In this case, an indicator that encompasses light, canopy volume and below ground competition could be more interesting and robust in the future.

1.2. Impact of shade on apple trees morphology, architecture and phenology

Light has been reported to be essential to achieve good yield in apple trees orchards (Palmer *et al.*, 2002) but is only one factor among others that could act in these complex environments. Our study revealed that apple trees expressed shade adaptation traits that involved the tree trunk morphology, architecture and their capacity to bear fruits. The number of ramifications was positively correlated to light quantity received by the tree but the proportion between long and short shoots stayed the same on the second and third year after plantation. This not only impacted the apple tree leaf area but also the proportion of flower clusters, which ultimately could impede the tree capacity to produce fruits.

Reducing light intensity decreased branching in the two studied years confirming the phenomenon of increased apical dominance under shade in general (Smith and Whitelam, 1997) and especially in the apple as observed on 'Ginger Gold' cultivar (Miller *et al.*, 2015). As expected, apple tree specific leaf area (SLA) was higher in shade (Gommers *et al.*, 2013; Ballaré and Pierik, 2017) which is an adaptation to maximise carbon gain per unit of leaf mass (Evans and Poorter, 2001). In our study, changes in SLA were not enough to compensate for light reduction as leaf area and trunk section area were significantly reduced. Furthermore, while a higher SLA helps intercept more light for photosynthesis, it is also related to a decrease of leaf thickness which in turn would affect leaf ontogenic resistance to apple scab (*Venturia inaequalis* (Cooke) Winter) and to herbivores in non-resistant cultivars (Ballaré and Pierik, 2017). This suggests that further studies should investigate relationships between these shade effects-mediated changes in leaf morphology and pest and disease symptoms.

Another common and observed shade avoidance trait is stem elongation (Smith and Whitelam, 1997; Valladares and Niinemets, 2008; Ballaré and Pierik, 2017). Our results suggest that it is the whole trunk geometry that is affected by light conditions rather than the shoot primary growth and that those differences appear when secondary growth occurs. One possible reason for these changes in trunk morphology could be linked to carbon assimilation and allocation. Photosynthetic rate is reduced in shade (Evans and Poorter, 2001) leaving less carbohydrates for the different sinks. Carbon allocation has been shown to be modified in shade avoidant species (i.e. root/shoot ratio) (Gommers *et al.*, 2013; Yang and Kim, 2019) and apple tree in shade could prioritize other sinks such as shoot and root primary growth over secondary growth. Relative growth rate of bourse shoots was also affected by light intensity. From three to five weeks after full bloom the majority of carbohydrates fixed by rosette (i.e. bourse leaves) and bourse shoot leaves are exported to fruit (Fanwoua *et al.*, 2014). While it has been reported that shade can delay partitioning in apple trees between shoots and fruits (Corelli Grappadelli *et al.*, 1994), the reduction of photosynthetic rate might have exacerbated local competitions for carbohydrates and therefore impeded the growth of bourse shoots. Shade tolerant species are expected to have a higher relative growth rate in shade (Valladares and Niinemets, 2008; Gommers *et al.*, 2013) suggesting that our plant material is not tolerant to shade. Looking at the different traits, it is hard to conclude on an avoidance or tolerance strategy concerning apple tree towards shade. A next step would be to study complementary traits (e.g. fruit growth) and validate the hypothesis of a modification of carbon allocation between compartments as well as to explore the genetic variability of these traits.

In a fruit tree based agroforestry system, the aim is to produce marketable fruit so there is a real incentive to study if and how shade will affect the reproductive strategy. We studied two essential steps: floral initiation and fruit set. The latter is the first hurdle that will determine yield during a growing season. Flowering has been reported to be accelerated

(Smith and Whitelam, 1997) or delayed (Lorenzo *et al.*, 2019) by shade depending on the experiment and plant material. Our study partially disagreed with these results. We showed that if the date of budburst was not related to light intensity, perhaps because walnut trees being a late leafing species differences in light conditions (due to trunk and branches) are less marked at that date. The phenology was modified around full bloom. In 2018 and 2019, flower clusters in shade lost their petals prematurely compared to flower clusters in full light. This could lead to a shorter pollination window, less attractiveness to pollinators (Moyroud and Glover, 2017) and could potentially affect negatively fruit set. However, in our study, light intensity did significantly reduce fruit set rate in full light conditions compared to shade, suggesting that the petal fall had a limited impact on fruit set rate. Shade even promoted fruit set rate in the apple tree, but this result could be a consequence of a lower fruit load at the whole tree scale.

A decrease in light intensity, whether natural or artificial, has a negative impact on floral initiation (Jackson and Palmer, 1977; Smith and Whitelam, 1997). Floral initiation is a complex phenomenon but is known to be correlated to the leaf area of the previous year (Lauri and Trottier, 2004; Buban and Faust, 2011; Belhassine *et al.*, 2019). In our study, a reduction of ca. 30% of light intensity significantly reduced the leaf area for both years, 2018 and 2019, and the number of flower clusters only in 2019. Although our study was carried out only over two years, this suggests that differences between apple trees in different light conditions will be more marked with aging. The lack of light could also extend the period before the apple trees enter a full reproductive stage which, in turn, would influence its flowering pattern (Costes and Guédon, 2012). However, the relation between leaf area of the previous year and the number of flower clusters in the current year was not modified between full light and moderate light. These two variables were positively correlated in 2019 and 2020. Moderate shade slows down the growth and development of the tree but does not seem to affect its fruiting capacity relative to vegetative development. Several studies have reported that there is usually enough non-structural carbon (NSC) to maintain a normal growth under reduced light conditions (Gruber et al., 2011; Kannenberg et al., 2018; Klein et al., 2014; Korner, 2003; Maguire & Kobe, 2015; Zhang et al., 2015) suggesting that shade and competition for water and nutrients were responsible for a reduction in apple trees growth and development. However, drought stress can modify allocation strategy between above and below ground compartment and result in lower NSC concentration (Gruber et al., 2011; Kannenberg et al., 2018; Zhang et al., 2015). Having no evidence that the concentration of starch and NSC were similar we could face a carbon depletion every year resulting in an increasing impact on the apple trees. More years of study will be needed before concluding but these results are promising for the future of apple tree based agroforestry systems.

However, when there was a reduction of ca. 65% of light intensity, floral initiation was greatly impeded. Apple trees had a significantly lower leaf area and less flower clusters in 2018 and 2019. In 2019, there was an average of only 10% of flower clusters on apple trees. Furthermore, there was no correlation between leaf area of the previous year and the number of flower clusters of the current year for trees in low light, suggesting that there are other signals linked to light intensity that prevented floral initiation. However, even a moderate light reduction and a modification in light quality could have a cumulative effect on apple tree development and fruit production (Morandi *et al.*, 2011; Bastías and Corelli-Grappadelli, 2012; Miller *et al.*, 2015; Lopez *et al.*, 2018) which should be investigated over a longer study period.

1.3. Shade as a tool to limit transpiration during summer and to buffer extreme heat

Some studies have emphasized on the interest of using shade or hails nets during critical period to mitigate extreme climatic events (Nicolás *et al.* 2005; Girona *et al.* 2012; Lopez *et al.* 2018). Our study revealed that the mean daily sap flow density (i.e. sap flow per unit of sap wood area, J_s) was not affected by environmental conditions but water use and transpiration per unit of leaf area were. Water Use (WU) and transpiration per unit of leaf area (E_l) were negatively impacted by shade. However, J_s dynamics (i.e. daily dynamic measured hourly) were affected by the environment especially during the summer. Apple trees in moderate and low light were less responsive to an increase in the vapour pressure maximum daily value (D_{max}) compared to apple trees in full light. These results suggest that shade or proximity to walnut trees limited the maximum value of J_s in the morning. However, air D_{max} is different from leaf to air D_{max} which is most probably lower in agroforestry.

As there was no effect of light conditions on apple tree J_s over the growing season differences in water use were due to differences in apple tree morphology and architecture. However, apple tree architecture and development were affected by light environment (Pitchers *et al.*, 2021). Above ground dry biomass was reduced in shade during both years which probably is a consequence of a reduction in photosynthetic rate (Charbonnier *et al.*, 2017) due to light quantity being not enough to saturate the leaf photoreceptors. The fact that significant differences in above ground dry biomass disappeared between 2018 and 2019 could indicate that these differences could decrease with the apples trees ontogeny and their architecture getting more complex it is probably an artefact due to the presence of fruits. Indeed, in 2019 5 fruits per cm^2 of trunk cross section area were left when possible and apple trees in full light almost always reached the objective while apple trees in moderate light were very heterogeneous as apple trees in low light. The fruits were not taken into account in the aboveground dry biomass while they are a strong sink for carbohydrate resulting in under-estimating the above ground dry biomass for all apple trees (Palmer *et al.*, 2002). As they were bearing more fruits, the above ground dry biomass of apple trees in full light were consistently underestimated compared to apple trees in moderate and low light. This lack of carbohydrate also affected primary and secondary growth with sap wood area (SWA) and leaf area (LA) being significantly lower for apple trees in shade. This resulted in a significantly lower Huber value (ratio of sap wood area on leaf area) in 2019 for the apple trees in low light, which would result in a decreased capacity of stem to transport water to leaf (Carter and White, 2009). Transpiration per unit of leaf area confirmed this tendency, in full light apple trees had a higher E_l which could be related to an increase in water transport to leaf. The differences in SWA also led to differences in water use. While this could be interesting in case of water scarcity to save water, orchards are usually irrigated to maintain apple trees in optimal conditions. Furthermore, there was no difference in water use efficiency (ratio of water use on aboveground dry biomass) in 2018 and 2019, so there was a proportional decrease in above ground dry biomass with the decrease in transpiration. In this regard, shade can be used as a mean to face water scarcity by limiting transpiration and could be used as a tool to help growers face particular climatic hazards (Lopez *et al.*, 2018). Interestingly light use efficiency was significantly higher for apple trees in low light in 2018 validating results observed on alfalfa (Querné *et al.*, 2017). However, in 2019 there was the same trend but not significant in light use efficiency (ratio of quantity of PAR received on aboveground dry biomass, LUE) suggesting that LUE differences might disappear with apple trees ontogeny or that the balance between above ground and below ground dry biomass can shift between years.

As seen before, mean daily sap flow density over the growing season was similar for all environmental conditions in 2018 and 2019. However, differences were observed when looking at daily sap flow dynamics specifically during summer when D and reference evapotranspiration (ET0) were high. In 2018 and 2019, when D and ET0 increased, there was an increase in the maximum value of J_s that changed according to the light quantity. In a low light environment apple trees J_s maximum was lower compared to apple trees in moderate and full light conditions suggesting that they were either less sensitive to environmental variables, controlling their transpiration earlier or that leaf temperature being lower thanks to shade diminishing the evaporative demand. Therefore, agroforestry thanks to shade could act as a buffer facing extreme climatic events. For example, in June 2019 there was extremely high temperatures recorded in the south of France which caused sunburn damage on the apples (Figure 20).

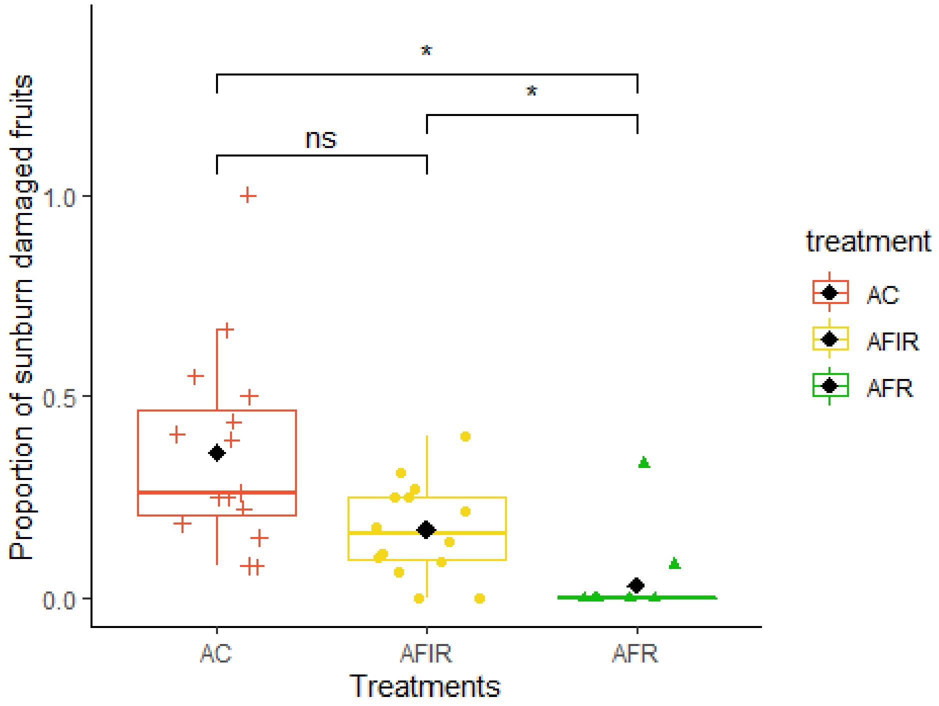

Figure 20: Proportion of sunburn damaged fruits after the extreme temperature recorded in June 2019 in Montpellier (AC: agricultural control; AFIR: agroforestry inter row; AFR: agroforestry row; *: $P<0.05$). A GLMM was performed following a quasipoisson distribution and with apple trees as the random effect.

There was a significative difference in the proportion of damaged fruit by sunburn between AFR (1.2%) and AC (26%). In the AFIR treatment, 17% of the apples were damaged by sunburn being not significantly different from AFR and AC. Our results suggest that agroforestry can protect apples from sunburn damage but only if there are close enough to the walnut trees.

We also observed that for values of D_{max} above 4, J_s in the morning peaked for apple trees in full light before being down regulated to the same level as apple trees in moderate and low light. This water transpired in the morning could represent a net loss in relative extractible water especially during summer when temperature is high and facing water scarcity. Lower J_s could also be interesting in an orchard facing water stress by limiting transpiration in the morning and maintaining relative extractible soil water high enough.

As said previously water use was impacted by light conditions but mainly as a result of differences in SWA rather than shade. However, we observed that correlations between leaf area and WU during summer were no longer significant in low light. This result could indicate a change of well-established relations like the number of flower clusters to leaf area (Pitchers *et al.*, 2021, 2nd chapter). Apple trees in low light could also experience shade at different moment during the day inducing different J_s patterns (Miller *et al.*, 2015) that could lead to difference in WU over a long period explaining why the relation was not significant.

E_l was impacted by shade and environmental variables. There is power relation between E_l and D_{max} or ET_0. The shape of the curve is similar for all light treatments and E_l increases with increasing value of D_{max} and ET_0. In the field, we were not able to evaluate the breakpoint after which we would observe a drop in E_l and if this break point would be different according to the light conditions. While the curves were similar among shade treatments as stated before there was a difference in the slopes. Apple trees in full light were more responsive to environmental variables and differences with apple trees in other light environments increased with increasing values of D_{max} and ET_0. This suggests that, as opposed to what was hypothesised, shade did not increase E_l during days with a high evaporative demand but that it even impeded them further. While there can be several reasons behind this behaviour we observed that cumulated E_l during the summer 2019 was well correlated to leaf area and the number of ramifications of apple trees. As leaf area increases and the architecture is more complex, apple trees transpiration per unit of leaf area increases. However, Lopez *et al.* 2018 have shown that shade can be beneficial in case of low water stress during summer but not when apple trees are well watered which was the case in our experiment. In this regard, agroforestry could have a positive effect on apple trees functioning in case of water stress. Apple is generally considered as isohydric (Jones, 2013), with strong stomatal control as opposed to anisohydric species with a weaker stomatal control upon water scarcity (Tardieu and Simonneau, 1998). Thus, it would be necessary to create different gradient of water stress to fathom the potential benefit of agroforestry in regard to the water scarcity issue and fathom how shade and water scarcity interacts in this particular context. Especially when considering that both genetic variability and phenotypic plasticity are involved in the range of iso-anisohydry morphological responses of apple trees to contrasted drought conditions (Lauri *et al.*, 2016).

1.4. Potential of AT-AFS

This work tried to highlight the potential of AT-AFS in the Mediterranean basin by studying the impact of an increasing competition on the architecture, morphology and water use of apple trees (Figure 21). This work showed that under low light conditions negative interactions are seemingly too strong and therefore, limit floral initiation and so, apple trees ability to bear enough fruit. However, a moderate reduction in light quantity (35% in this work) allowed to still reach our aim of 5 fruits/cm² of trunk cross section area in some cases which is a promising result when looking at the potential of AT-AFS. In our study it is still important to note that no positive effects induced by the presence of walnut trees were confirmed on apple tree architecture, morphology or water use. To precisely evaluate the potential of AT-AFS other aspects should be evaluated like pest regulation, generated biodiversity, leaching of nitrates, soil carbon in other things and compared to conventional apple orchards in organic farming or integrated fruit production and different pedoclimatic conditions.

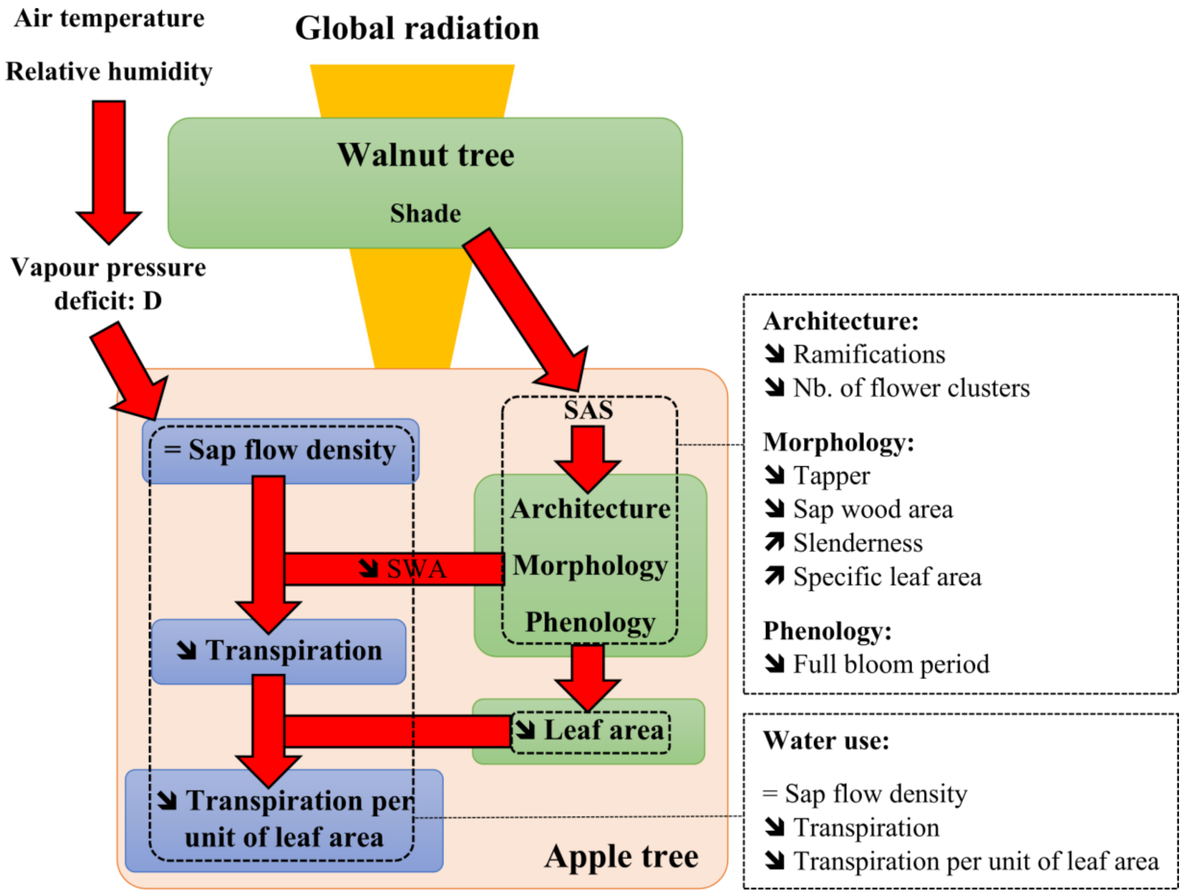

Figure 21: Principal results of the impact of shade in an AT-AFS represented in the conceptual framework around which the study was realised to estimate if and how an AT-AFS impacts apple trees development and water use (SAS: Shade avoidance/adaptation syndrome). The correlation between leaf area and the number of flower clusters of the next year was also modified by an increase in shade (see chapter 3).

2. Limits of the study

This work has created basic knowledge to understand the effect of plant interactions in an AT-AFS, showed that light is a main environmental factor to AT-AFS and paved the way to help optimize such agrosystem. Apple trees were still young and were not in their full reproductive phase, but our objectives were not to quantify fruit production on mature trees but to analyse the architectural and functional establishment of apple tree which is important since tree shape and general volume is established in these very first year. In agroecological mixed orchards, the time required to reach a productive orchard could be increased, delaying the return of investment for growers. There is still a lot to learn from this experiment as the fruiting patterns of an adult tree might be different to what was observed so far. Looking at regularity of production along the light gradient will also be critical as irregular bearing has been a main concern for research and growers in the past decades. Furthermore, two years of measurements are probably not enough to conclude on apple tree behaviour along a gradient of competition for light. As we have seen, patterns and significant differences in architectural and morphological traits vary from one year to the other. While, as aforementioned, it could be linked to a delay before entering their full reproductive phase it could also be linked to other factors inherent to our plot and its pedoclimatic conditions.

As mentioned, AT-AFS is a complex agrosystem in which light is only one factor among others that could act in these complex environments. For example, apple trees have been reported to be sensitive to juglone, the phenolic compound that is the agent of *Juglans* spp. allelopathy (Galusha, 1870; McWhorter *et al.*, 1874 cited in Jose, 2011). Soil under 10-year-old black walnut trees (*Juglans nigra*) alley cropping system can have significant amounts of juglone if release rates are greater than the abiotic and microbial transformation rates (von Kiparski *et al.*, 2007). However, the concentration of juglone drops significantly with distance from the walnut tree row (Jose and Gillespie, 1998a) and the highest concentrations of juglone measured do not exceed the concentration inhibition threshold of crops typically considered for intercropping (Jose and Gillespie, 1998b). A more recent study showed that there are several processes that can be altered by lower concentration of juglone which can limit water and nutrient uptake (Hejl and Koster, 2004; Böhm *et al.*, 2006). While we mentioned that it is safe to hypothesise that apple trees planted on the agroforestry inter-row and the control are probably not influenced by walnut allelopathic effect and it could be a confounded factor for walnut and apple trees planted on the same row it would still be important to measure the quantity of juglone in the soil. Indeed, after walnut tree thining in

2004 some apple trees have been planted on the exact same location and have shown problem in their development. We have no evidence that it is linked to residual juglone still present in the soil and therefore this should be investigated to ascertain that apple tree can grow in a walnut tree AT-AFS. However, our walnut trees are a hybrid of *Juglans nigra* and *Juglans regia* and further studies should investigate if the quantity of juglone exudated are similar.

We also noticed during the third and fourth year that there might have been an incompatibility between Dalinette cultivar and the G202 rootstock. Several apple trees broke at the graft point and it did not seem related to their size or planting conditions. Therefore, amongst the other environmental factors that influenced apple trees growth and development the graft could also have had an influence that we did not predicted or accounted for. To our knowledge, this phenomenon has been described in *Prunus* tree (Errea *et al.*, 1994; Gainza *et al.*, 2015) but not *Malus*.

Finally, I think it is important to mention that our results are very dependent on our pedoclimatic conditions and therefore cannot be generalized as it is usually the case for system experimentations. Usually, the aim behind these experimentations is to evaluate the capacity of a cultivation system to satisfy given objectives while factorial experiments are used to study and understand the effect of one or a few factors and their interactions taken in isolation, all other things being equal, on one or more dependant variables (Deytieux *et al.*, 2012). While the first approach aims to evaluate a global system with regard to defined objectives the second can help improve agrosystems by improving a technical aspect or a decision rule (Deytieux *et al.*, 2012). In this work we studied how a gradient of increasing competitions would impact apple tree growth and development in an AT-AFS by considering light as the limiting factor and the one explaining factor. This implies that we considered working in a ‘factorial’ experiment to establish decision rule to help prototyping AT-AFS. While our results may able us to establish basic guidelines one should keep in mind that these results are very dependent to our experimental plot and that it would ask for a network of plot in different pedoclimatic conditions if we wanted to generalize our results.

3. Perspectives

This work, in my opinion, has open the path to a more global reflexion on AT-AFS and from an applied point of view, brings some practical aspects that should be better taken in consideration to design optimized AT-AFS. For example, we established that there is a minimal PAR values below which the target apple tree growth and flowering is considered as insufficient from an agronomic point of view. This suggests that under a certain threshold, approximatively 70% of incoming PAR in our case, the capacity of apple trees to bear enough fruit is impeded. Considering that maximizing PAR interception is not always the best solution because of light damaging effects on leaf functioning and fruit quality (Grappadelli and Lakso, 2007) it is also likely that the effects of PAR on the three apple tree variables considered here (i.e. number of ramifications, flower clusters …)are asymptotic instead of exponential. In this case, a maximal PAR interception threshold beyond which the target tree functioning is not improved or is even impeded should also be considered. Designing an apple tree based agroforestry system should then consider this optimal PAR values range, between minimal and optimal values, that should be determined more accurately depending on the apple genotypes and pedoclimatic conditions.

With that in mind, we can now emphasize on the fact that there was little management of apple and walnut trees during our experiment to limit the number of varying factor and avoid confounding factors as management would influence apple tree architecture and bearing pattern. The actual bearing pattern of apple trees results both from its endogenous potential and the way it reacts to its environment and the training and pruning procedures (Breen, 2016). A next step would then be to work on apple tree training system and study the interaction between environmental variables induced by the presence of neighbouring plants and the training system on the bearing pattern. Furthermore, the timber trees occupying the upper strata could also be managed to optimize the quantity of light reaching the plants in the lower strata in the value range of PAR defined earlier.

Testing different apple genotypes and scion/rootstock combinations is also an important step. Cultivars are always evaluated in optimal conditions with the idea that there would be planted in a conventional orchard and not in low input conditions. This subject has been controversial for several years in the scientific community that insists on the fact that varieties should be evaluated in the conditions they have been selected for. For example, a variety of durum wheat that had been selected for organic farming as then been evaluated in conventional conditions and, as it performed less than then other varieties, was rejected by official institutions. If we want AT-AFS to be optimized, it is important to breed apple tree cultivars that perform well in low input and high competitive environment especially in low light environment (Desclaux *et al.*, 2009). In our experiment, we chose a cultivar that has been reported to perform well in OF and produces red apples hoping that it would perform well in AT-AFS and evaluate if there were fruit colouring issues in shade. However, testing other genotypes and different combination of scion and rootstock is an essential next step to help optimize AT-AFS and study them in different pedoclimatic conditions.

I would like to emphasis the fact that AT-AFS has potential and could even produce enough fruit in quality and quantity and still offer other ecosystem services if the means to help develop such agrosystems are provided. There are a lot of different pathways that can lead to a more sustainable agriculture and I am convinced than AT-AFS can be one solution among others to revisit fruit tree production and orchard management. However, as it is an agrosystem like no others, to be successful it would need more research and developments in breeding, management and training to be able to have enough knowledge to optimize FT-AFS. I hope that this work could help convince political deciders that FT-AFS is worth investing into it. Pushing further this study by including different genotypes and management practices (e.g. pruning, pollarding walnut trees, coplanting to limit competition for light in the first years, …), will provide valuable insights in fruit tree based agroforestry systems in temperate climate.

4. Conclusion

This thesis work has provided knowledge on the impact of an increase competition for light mainly and nutrients on apple trees in an agroforestry system.

The first objective was to characterize the environment of the apple trees by using a distance-dependent model of canopy crowding (NCI) or quantifying the photosynthetically active radiation (PAR) reaching the crop. We confirmed that apple tree vigour is negatively correlated to an increasing number and size of neighbour trees and positively correlated to light quantity. Furthermore, in the specific context of our study, we showed that considering PAR and aboveground interactions only, explained a higher proportion of the different traits measured to select our indicator. Aboveground interactions were prevalent likely because of irrigation and fertilization that could minimize underground interactions if roots of apple trees and walnut trees occupy the same area for which we have no evidence. In other words, the management of the experimental plot participated in shifting the balance between above and belowground competition compared to a 'natural' ecosystem. We then chose to analyse apple tree architecture, morphology, phenology and water use considering PAR received by the apple tree as the main discriminating variable.

The second objective was to determine what traits were affected by a gradient of light reduction in the apple tree, and more specifically, whether and how shade impacted its flowering and fruit-set pattern. We quantified morphological and architectural traits, at shoot and whole tree scales respectively, as well as phenological traits of flower clusters. The number of growing shoots and the leaf area was reduced by shade even if specific leaf area increased with increasing shade. Shade did not modify primary growth but did decrease secondary growth. It made apple trees in shade slender, with a lower taper and reduced the number and proportion of flower clusters. The correlation between floral initiation and leaf area was high both in full and moderate light but not for apple trees in low light. Shade did not impact the date of bud burst and the early phenological stages of flower clusters, but it reduced the number of days at full bloom. Our results suggest that while the architecture of apple trees is modified by a reduction in light intensity, it is not until a reduction of 35% that the capability to produce fruit is impeded. These results could help optimize the design of apple tree based agroforestry systems**.**

The third objective of this study was to quantify sap flow density (J_s), water use and transpiration per unit of leaf area (E_l) of apple trees, to determine how the light environment influenced J_s and E_l, and to study relations between apple tree architecture at the tree scale and J_s or E_l. Benefiting from a favourable microclimate and less excessive radiation, we expected apple trees in shade to maintain their stomata open longer than apple trees in full light. Therefore, J_s during specific day, i.e. high vapour pressure deficit (D) should have been higher for the apple trees in agroforestry compared to apple trees in full sun. Our results indicated that the apple tree adapted to shade through morphological adaptation (SLA, Huber value) and that there was no benefit of shade and the microclimate generated by the proximity to walnut trees on water use and transpiration per unit of leaf area. However, by limiting their transpiration in shade during the summer apple trees could help maintain soil water content to face water scarcity.

In the light of these results, to be successful, apple trees in an AT-AFS with alley cropping should not be planted at less than 6.5m from timber trees so to limit the impact of shade and competition for water and nutrients. In this latter case, apple trees still benefit from the microclimate generated by the timber trees on their transpiration and manage to produce a satisfactory number of fruits in quantity to support production. Closer to timber trees, we have seen that strong shade had a negative impact on apple trees. A solution to mitigate the impact of shade during the apple trees establishment could reside in co-planting the timber tree and the apple tree so during the first years the timber trees do not dominate the apple trees. Finally, even if the production service might be reduced compared to traditional orchards, FT-AFS are interesting for other services they may provide provided other plants than only apple and timber trees are integrated in the system such as herbaceous to fulfil other services (pest regulation, nitrogen cycling…; Lauri and Simon, 2019). While they might not all be of the same importance, depending on the actor, it is necessary to consider the system globally and study every aspect of it before concluding on their interest.

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ANNEXE 1

Growing agroforestry systems with apple in Montpellier-Mediterranean – preliminary results on the influence of adult walnut trees on growth and branching of two-year-old apple trees

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Abstract

Apple cultivation has evolved tremendously in the past decades to increase tree productivity and fruit quality. However, this was achieved at the cost of an increasing dependence on external inputs such as water, fertilizers and pesticides. This dependence is now questioned because of the generated environmental pollution and health issues. Different solutions have been considered to reduce this dependence including more efficient practices that improve resilience of agricultural systems to external pressures (climatic disturbances, new diseases, economic crises). To reduce pest and disease pressure, to improve resources use and to buffer extreme climatic events, agroforestry systems (AFS), i.e., the association, in one field, of tailored perennial and annual crops and possibly animals, are proposed as a way to use positive interactions between various plants. In temperate climate, AFS associate a tree stratum and a (or several) crop stratum, e.g., fruit trees and vegetables. An original AFS located in southeastern France, characterized by a Mediterranean climate and composed of mature walnut grown for timber, apple trees in an intermediate vertical stratum, and alfalfa at the lowest stratum, is being used to study the impact of an AFS on apple trees. Comparing apple trees in the AFS and in full sun, we first showed a significant effect of walnuts on the below-canopy microclimate reducing incoming global radiation and acting as a buffer on temperatures surrounding the apple trees. Secondly, AFS significantly altered the apple tree stem geometry (lesser tapering), growth dynamics (more growth cessation) and morphology (higher individual leaf area and specific leaf area). Results will be discussed with regard to the putative long-term effects of agroforestry conditions on the apple tree architecture and fruiting.

Keywords: *Malus* × *domestica*, tree architecture, microclimate, hydric potential, specific leaf area

INTRODUCTION

Current agriculture has to face new ecological and societal challenges by increasing its production while addressing environmental concerns. Different solutions have been considered including more efficient practices that improve resilience of agricultural systems to external pressure (climatic disturbances, new diseases, economic crises) (Foley et al., 2011). While apple cultivation has evolved tremendously in the past decades to increase tree productivity and fruit quality, it is highly dependent on external inputs such as water, fertilizers and pesticides. This dependence is now questioned because of the generated environmental pollution and health issues.

Agroforestry systems (AFS), i.e., the association, in one field, of tailored perennial or annual crops and possibly animals, are proposed as a way to use positive interactions between various plants to reduce pest and disease pressure, to improve resources use and to buffer extreme climatic events. In temperate climate, AFS associate a tree stratum and a (or several) crop stratum, e.g., fruit trees and vegetables. In this way, AFS are interesting because they increase the production on the same land area, e.g., a higher land equivalent ratio (Gliessman, 1985), while meeting environmental concerns. Land equivalent ratio is defined as the relative land area required as sole crops to produce the same yields as intercropping. However, to design efficient and effective AFS that will meet expectations, they still need to be studied thoroughly.

An original AFS was designed in south-eastern France under Mediterranean climate and composed of mature walnut grown for timber, apple trees in an intermediate vertical stratum, and alfalfa at the lowest stratum. This aims to be a long-term study on the apple trees architecture, the regulation of pests and disease, the fruit quality and the bearing pattern. It is considered that, at any given stage, tree architecture and functioning reflects internal competitions for photosynthetic assimilates (Aguirrezabal et al., 1993) that will potentially vary depending on the intensity and location of the trophic competition (above- and belowground). This would apply to our case between the apple and the other plants of the system, here walnut and alfalfa. Therefore, our objective was to decipher the respective effects of competition for light on the apple tree development during its second year after the plantation by comparing apple trees in AFS and in full sun. Results will be discussed with regard to the putative long-term effects of agroforestry conditions on the apple tree architecture and fruiting.

MATERIALS AND METHODS

Study site

The study site is located on the "Domaine de Restinclières" (municipality of Prades-leLez, Hérault, France, 43°42'12.168"N; 3°51'29.872"E – https://umr-system.cirad.fr/en/theunit/research-and-training-platform-in-partnership/restinclieres-agroforestery-platformrap). Apple trees (*Malus domestica* 'Dalinette' grafted on semi-dwarfing G202 rootstock) have been planted as an intercrop in March 2016 on a plot with walnut trees (*Juglans nigra* × *Juglans regia* NG23) planted in February 1995 and intercropped alfalfa (*Medicago sativa*) sowed in 2012 (Figure 1).

Figure 1. View of the experimental design with (a) walnut trees, (b) apple trees on the row and the inter-row and (c) alfalfa intercropped. Two rows of walnut trees are 13 m apart, with 4 m between trees with possibly 1 or more walnut trees missing. A row of apple trees was planted at 6.5 m in between two rows of

> walnut trees. In all cases, apple trees are distant of 1.3 m along the row. Rows of trees are oriented E/W.

Apple trees are planted in two blocks (*n*=83 and *n*=65, respectively) and three treatments: i) in full sun (agricultural control, AC, *n*=26), ii) in agroforestry on the inter row (AF_IR, *n*=85), e.g. between two rows of walnut trees, and iii) in agroforestry on the same row as walnut trees (AF_R, *n*=37).

The apple trees are managed according to the organic technical specifications. Woodchips are spread in order to control weeds on the row. Drip irrigation and fertilization were provided following technical advice by an extension service.

Characterization of aboveground environment

Hemispherical pictures were taken with a fish-eye lens to measure the gap fraction (GF) above the studied apple trees. We took pictures before and after walnut trees bud burst to see how the difference in GF for apple trees evolved throughout the growing season. Ten pictures per treatment were taken next to the apple tree at pre-dawn to avoid direct sun light. The images were then threshold in black and white using PiafPhotem before being processed in PiafLA (https://www6.ara.inra.fr/piaf/) to obtain the standard overcast sky value.

Apple trees development

Before 2017 bud burst, the length and diameter of the apple trees trunks, hereafter referred to as stems, were measured using a measuring tape and an electronic calliper, respectively. For diameter, as trunk cross-section is not perfectly round, we took the average of two measurements perpendicularly done with the calliper. These diameters were measured on an internode at the base and the apex of the 2016 stem. To decipher differences in geometry after one year among the apple trees in our different treatments we used classical forestry indicators, slenderness and taper. Slenderness is the ratio of the stem's length divided by the mean diameter and is used to evaluate the elongation of the stem, while taper is the difference of the diameter between the base and the apex divided by the trunk's length, i.e., the conicity of the stem.

The growth of the apical shoot in 2017 was followed throughout the growing season by measuring the length every week with a measuring tape and counting the number of new leaves.

The specific leaf area (leaf area/dry mass ratio) was calculated on 15 trees in each treatment using 2 leaves tree^{-1}. The leaves were collected at pre-dawn on site and put into plastic bags with humid paper. The plastic bags were then placed in an ice compartment until being processed at the laboratory. The leaf area was measured using a scanner and the software WinFOLIA®. Once scanned the leaves were placed individually in a paper bag and in an oven at 60°C for 48 h. Once dried each leaf was weighted on a scale with a precision of 0.01 g.

Data analysis

Statistical analyses of variance were performed with R software for most of our collected data when the hypothesis of normal distribution and homoscedasticity of the residual were confirmed. If not, we used a non-parametric, Kruskal-Wallis test by ranks (Chan and Walmsley, 1997). The effect of the block was tested each time but was never significant. Standardized major axis method was used to analyze data on the stem geometry, slenderness and taper as well as allometric relationship between stem length and diameter on log transformed data. In all cases threshold for significant difference was set at 0.01.

RESULTS

Influence of the walnut trees on the aboveground environment

The GF showed that there was a difference in the direct light environment and the global radiation received by the apple trees in the different treatments (Figure 2). Before walnut trees budburst (April) GF varied from 0.98 for AC to 0.83 for AF_R. AF_IR was at

0.93. While significant, these differences were still relatively small. When the walnut trees were fully foliated, significant differences among treatments increased from 0.97 for AC to 0.36 for AF_R treatment and 0.73 for AF_IR.

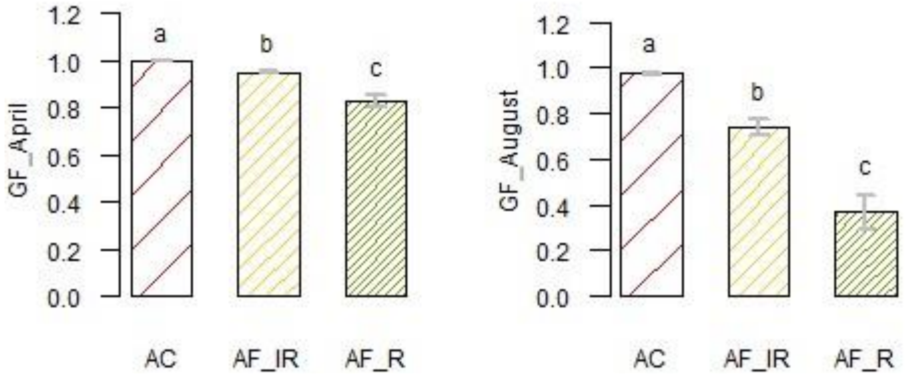

Figure 2. Results on values of the gap fraction (GF) calculated by PiafLA software for 30 apple trees before walnut trees budburst (April) and at full foliation (August). Ten pictures in each treatment were analyzed (AF_IR: Agroforestry inter-row; AF_R: Agroforestry row; AC: Agricultural control). Data are means ± SD. Letters above bars are statistical groups (Kruskal-Wallis test; α=0.01). **Apple trees growth and development**

1. Stem geometry.

There is a significant difference for taper between apple trees in AC and apple trees in AFS (Figure 3). In AC apple trees lose more than 0.04 cm of stem diameter cm^{-1} of stem while in AFS apple trees lose 0.035 cm of stem diameter cm^{-1} of stem. For slenderness we used a standardized major axis (SMA) regression on Log_{10} transformed data between average stem diameter and stem length. We have shown that the slope being significantly different from 1 there was an allometry for apple trees in AFS (Table 1). In other words, the stem length increases faster than the stem diameter therefore suggesting that for the tallest tree there is a difference in slenderness among our treatments.

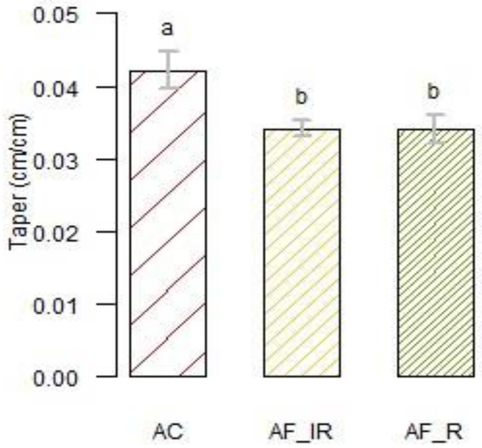

Figure 3. Tapering (cm loss of diameter cm^{-1} of stem), of two-year-old apple trees in 3 different treatments (AC; AF_IR; AF_R). Data area means ± SD. Letters above bars are statistical groups (Kruskal-Wallis test; α=0.01).

Table 1. SMA regression between average stem diameter and stem length on Log_{10} transformed data on one year-old apple trees in an AFS (n: sample size; slope: slope of the relationship; p-value: probability associated to isometries (null hypothesis H_0=1); Type of relationship: isometry (the proportional increase of

the two variables) or slenderness (the increase of stem diameter is less than the increase of stem length).

Treatment	*n*	Slope	p-value	Type of allometry
AC	21	0.8	0.151	Isometry
AF_IR	74	0.59	<0.01	Slenderness
AF_R	26	0.62	<0.01	Slenderness

2. Growth dynamics.

There were no significant differences in the growth dynamics of stems across the three treatments (data not shown) but there were differences in the proportion of temporary stem growth cessation (Lauri et al., 2016) (Figure 4). During the first eight weeks there were no differences in the proportion of stem growth cessation (less than 10%) between the apple trees in the three treatments. When shading by the walnut trees became maximum and the end of June, there was clearly a difference between AC and both AF_IR and AF_R. While the apple trees in AC did not suffer any increase in stem growth cessation there was over 20% of apple trees that did in the AF_IR and AF_R. The proportion of stem growth cessation was stable for four weeks in the different treatments before increasing in two weeks to approximately 70% for AC and 80% for the AF_IR and AF_R. The last week, there was a regrowth after growth cessation for the apple trees in AC and kept relatively stable for AF_IR and AF_R.

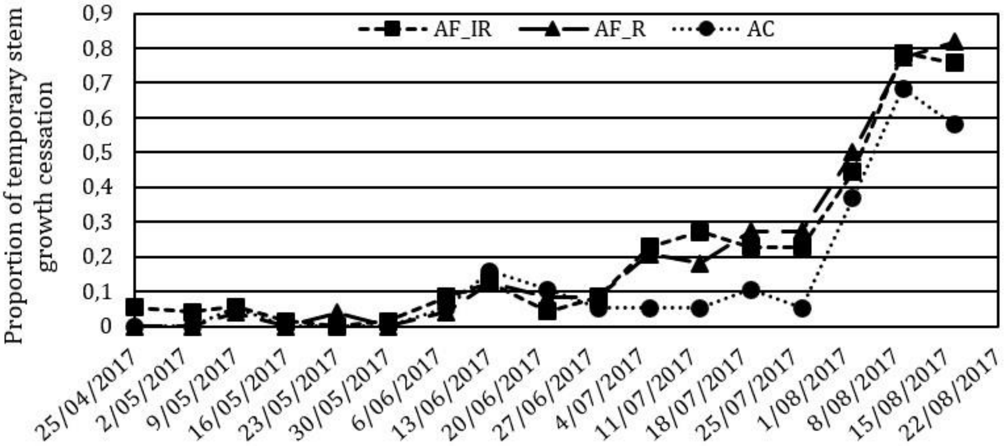

Figure 4. Proportion of stem growth cessation of two-year-old apple trees in three different treatments (AC; AF_IR; AF_R). We considered temporary stem growth cessation when there was no leaf establishment and a stem elongation inferior to one centimeter over a week.

3. Leaf area and specific leaf area.

There were significant differences among treatments for both mean leaf area (LA) and mean specific leaf area (SLA) at the leaf level (Figure 5). LA of AF_R (54.8±17.4 cm^2) and AF_IR (41.7±8.2 cm^2) was significantly higher than LA of AC (32.4±5.6 cm^2). SLA of AF_R (133±15.7 cm^2 g^{-1} dry matter) was significantly higher than SLA of AC (108±14.4 cm^2 g^{-1} dry matter) with SLA of AF_IR in intermediate position (123±20.4 cm^2 g^{-1} dry matter).

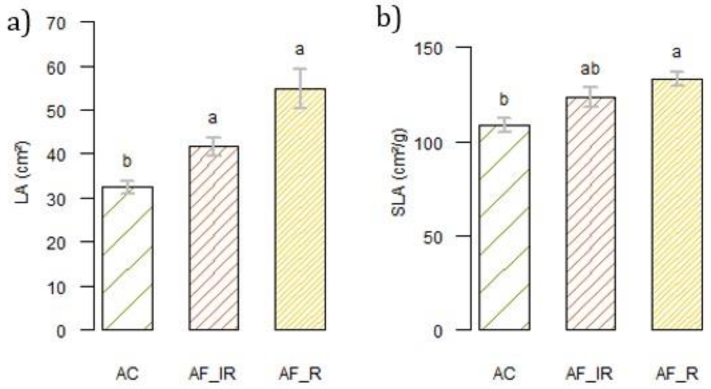

Figure 5. Leaf area (a) and specific leaf area (b) analyses of 2-year-old apple trees in an AFS in 3 different treatments (AC; AF_IR; AF_R). Data are means ± SD. Statistical groups according to Kruskal-Wallis test (α=0.01).

DISCUSSION

The presence of walnut trees i) buffers daily temperature variations (minus 0.5°C in AF_IR and AF_R, data not shown), and ii) reduced by 50% the incoming light and incident radiation on the apple trees. While the apple trees had the same growth rate (identical plastochron), the competition with walnut trees induced differences in growth dynamics, stem geometry and leaf morphology (Figure 6).

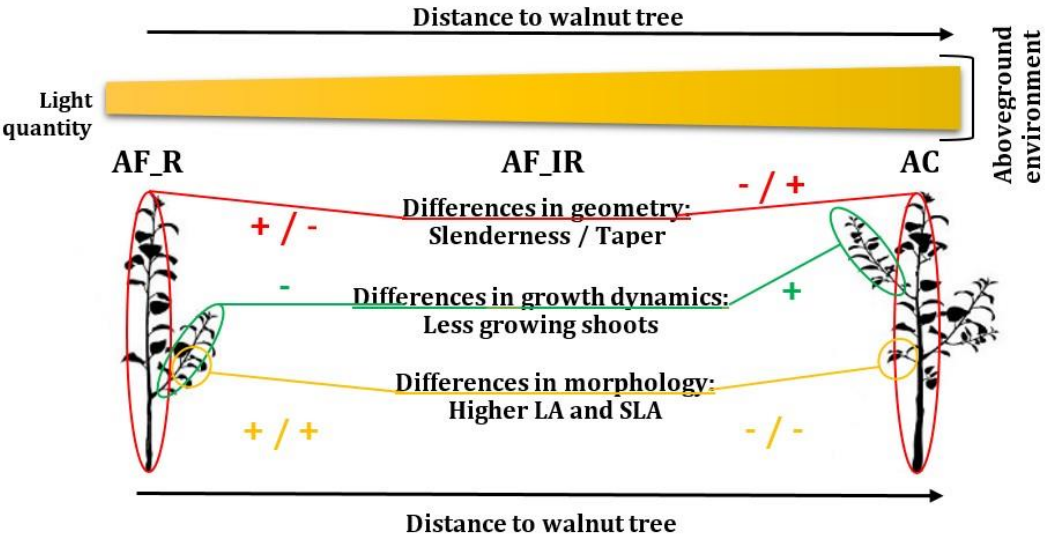

Figure 6. Representation of the apple trees and their environment changes in relation to their distance to the walnut tree. The changes between our treatments were significantly different between AF_R and AC only.

In a shaded environment where light capture is essential, we can expect longer stems and internodes (Kami et al., 2010), leaves with a high specific leaf area (SLA), an increase in leaf area and reduction in root/shoot ratio (Lambers and Poorter, 1992). Furthermore, these phenotypic adaptations are most apparent in species of unshaded or lightly shaded environment (Grime, 1977). As shown in our study, the apple tree in AF_R tend to have smaller trunks (in length and diameter) with a trend toward slender and less conical shape, higher LA and SLA. These results suggest that the apple tree is a specie of unshaded or lightly shaded environment. However, it would be necessary to compare different species, apple genotype and rootstocks to evaluate the degree of reaction of a given genotype/rootstock to a shaded environment.

The differences in growth dynamics that occurred at the beginning of July when the competition for light was maximum (i.e., walnut trees at full foliation) could have consequences on the growth of apple trees in AFS in the long term. Apple trees in AF_IR and AF_R compared to the apple trees in AC suffered from a precocious and longer stem growth cessation therefore leading to less vegetative growth over the season that can be accumulated over the years. Reduced leaf area coupled with stem growth cessation have been suggested to be a drought avoidance strategy and also illustrates an architectural escape strategy to stress (Lauri et al., 2016).

Shorter shoots and less nodes related to a smaller leaf area at the plant level are not compensated by higher LA at the leaf level and so less photosynthetic assimilates are produced. This lack of sugar synthesis and nutrients could limit flower induction of the apple trees in the AFS resulting in potentially less yield. But this could also limit biennial bearing as heavy fruit load inhibits floral induction and vegetative growth of the next year (Samach and Smith, 2013; Smith and Samach, 2013). However, before drawing any conclusions it is necessary to study how fewer floral buds paired with less vegetative growth acts on fruit load regularity.

Apple trees have been reported to be sensitive to juglone (Galusha, 1870; McWhorter et al., 1874; Jose, 2011), the phenolic compound that is the agent of *Juglans* spp. allelopathy. It has been reported that soil under 10-year-old black walnut trees (*Juglans nigra*) in an alley cropping system can have significant amounts of juglone because release rates can be greater than the abiotic and microbial juglone transformation rates (von Kiparski et al., 2007). The concentration of juglone decreases with distance from the tree row but is still detected to up to 4.25 m of the row (Jose and Gillespie, 1998a). The highest concentrations of juglone estimated approached but did not exceed the concentration inhibition threshold of crops typically considered for intercropping (Jose and Gillespie, 1998b). However, there are several processes that can be altered by lower concentration of juglone that can limit water and nutrient uptake (Böhm et al., 2006; Hejl and Koster, 2004). In our experiment it is not possible to decipher the effect of shade and accumulated juglone on the apple trees on the same rows as walnut trees. With the apple trees on the inter-row being at 6.5 m of the walnut, it seemed that juglone had a limited effect in this study.

CONCLUSIONS

This study showed that the apple trees adopted different growth strategies in relation to the modification in their aboveground environment. However, it is important to bear in mind that the end goal of the fruit tree is to bear enough fruit in quantity and quality to generate an income. Fruit set and early fruit development (leading to final fruit size and yield) rely on supply of carbohydrates to the reproductive organs which are sourced from reserves and current photosynthesis. During the first weeks after bloom, carbohydrates demand from developing shoots and fruit sinks is likely to be greater than supply, and shoot development is thought to have priority for limited carbohydrate supply over fruit development. This competition among sinks results in fruit abscission (Lakso et al., 1999). Ongoing studies aim at measuring the impact of differences in development and architecture on the proportion of floral buds and fruit abscission to estimate if the trees growing in AFS have the potential to reach satisfying yields.

ACKNOWLEDGEMENTS

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ANNEXE 2

A NEIGHBOURHOOD ANALYSIS TO CHARACTERIZE COMPETITION IN A MULTI-STRATA AGROFORESTRY SYSTEM OF TIMBER AND FRUIT TREES.

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A need to characterize competition in agroforestry systems

The Growing AgroForestry systems with Apple in Mediterranean climate (GAFAM) project looks at the growth and development of apple trees in a multi-strata agroforestry system where walnut trees planted in 1995 forms the upper stratum, four-year-old apple trees the intermediate stratum and sainfoin the herbaceous stratum (figure 1). However, analyzing multi-species, multi-strata agrosystems using a categorical variable, i.e. 'treatments', is not satisfying when looking at the heterogeneity within each 'treatment' and could possibly hide differences between treatment when using comparison of means statistical tests. Inspired by forest ecologists we developed a neighbourhood competition index (NCI) to characterize competition for each of our focal apple tree, competition for light being a key determinant of productivity here (Jucker et al., 2014).

Creating an indicator to suit our needs

Our NCI combines traditional distance-dependent crowding models (Canham et al., 2004 ; Fichtner et al., 2017 ; González de Andrés et al., 2018) in which the target tree growth and fruitng is analyzed as a function of the sizes and distances to neighbouring trees and a season-dependent canopy shading factor. For $i = 1, \ldots, n$ neighbours within a maximum radius (r=15 meters) of the target tree the net competitive effect of the neighbours on the target tree is given by equation [1].

$$[1] \quad \mathrm{NCI} = \frac{1}{\sum_{t0}^{t} GF} \times \sum_{i=1}^{n} \frac{\mathrm{CSA_i}}{\mathrm{distance_i}}$$

Where GF is the sum of the gap fraction (derived from hemispherical photography) during the focal tree growing season and CSA the cross section area at breast height of the neighbour *i*. The apple trees being fertilized and irrigated we hypothesized that the limiting factor would be light. Therefore, we only considered the walnut trees in the creation of our NCI.

Validation of our NCI

The apple trees are planted in three treatments, on the same row as the walnut trees (AFR), on the inter-row 6.5m away of two walnut row (AFIR) and furthest away of the walnut trees considered as our agricultural control (AC) establishing a gradient of competition with the walnut trees. We correlated the trunk diameter of the target trees with the NCI (figure 2a). The NCI we calculated explained 59% of the variation of the target trees trunk diameter diameter and 49% of the number of flower clusters on 45 apple trees (figure 2b).

Take home message

- ✓ When working in complex agroforestry systems using a continuous indicator based on a focal tree can complement analysis using only treatments.
- ✓ Improving distance-dependent competition models by using a gap fraction based NCI proves to be relevant to model tree architectural features.

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Figure 1: A view of the plot with sainfoin at the bottom, apple trees in the middle and hybrid walnut at the top.

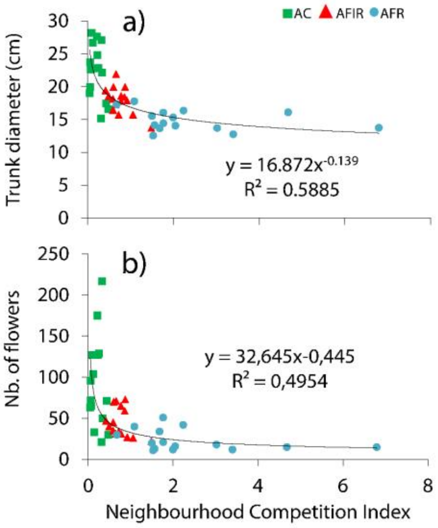

Figure 2: a) Correlation between the trunk diameter and our NCI for 45 3-year-old apple trees along a gradient of competition for light. b) One example of data analysis using the NCI. Here we correlated the number of flowers per tree for 45 apple trees with the NCI (AC: Agricultural Control, AFIR: AgroForestry Inter-Row, AFR: AgroForestry Row). NB: increasing NCI values means increasing competition.

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ANNEXE 3

Apple farming systems – current initiatives and some prospective views on how to improve sustainability

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Abstract

Apple cultivation has evolved tremendously in past decades. Both apple productivity and aesthetic quality of the fruit have been strongly improved resulting from genetic improvement, optimization of tree training and pruning, and orchard design and management. However, these improvements were also done at the expense of an increasing dependence on external inputs such as water, fertilizers and synthetic pesticides. This dependence is now questioned because of the generated environmental pollutions and health issues. In the last decades, an increasing amount of initiatives have been developed that open the way towards more sustainable apple production systems. Concepts as well as on-station and on-farm works are developed in various contexts such as 'integrated fruit production', 'organic farming' and 'agroecology' with the objectives to increase biological regulations of pests and diseases and/or to improve soil fertility. All together results point out the importance of diversifying resources and habitats for beneficial arthropods in the orchard and its vicinity to foster ecosystem services related to pest suppression and to adopt cultural practices enhancing soil fertility. They also indicate some practical guidelines consisting in a better management of grass alleys and lining hedgerows within and around the orchard, respectively. From a more prospective view and taking inspiration from tropical fruit-tree based agroforestry, these works suggest that combining apple trees with other herbaceous and woody plants with various uses (soft fruit, aromatic plants, etc.) opens to more resilient agroecosystems, possibly mitigating climate change. These works also enlarge our vision of the current apple orchard towards a multiproduction system including apple among other productions. From the 'plant science' point of view the idea to grow apple in agroecosystems challenges current knowledge of the plasticity of the apple tree physiology and architecture, and agronomic performance, in response to interactions with neighbouring plants. It also stimulates necessary collaborations with other research fields such as socioeconomics, for example on how the grower may handle those complex agroecosystems, optimize labour and valorize production.

Keywords: integrated fruit production, organic farming, agroecology, ecosystem services, agroforestry, climate change, socio-economics

INTRODUCTION

It is likely during the 1930s that apple was isolated from the traditional multispecies, often agrosilvopastoral, systems to be cultivated in monospecies fruit-tree orchards (e.g., in Europe; Herzog, 1998). Although statistics at the world scale need rigorous analyses and should be taken with caution, with for example a strong decrease of cultivation area from the middle on 1990s onwards (Figure 1a) that is not really consistent with changes in productivity (Figure 1b), some general statements can be made. In past decades, worldwide apple cultivation has undergone a tremendous increase in both cultivation area (from ca. 1.8 million ha in 1961 to ca. 7.7 million ha in 2016) and production (from ca. 17.2 million t in 1961 to ca. 133.8 million t in 2016) (Figure 1a). In the same time range mean productivity has increased from 5.9 to 15.2 t ha^{-1} (Figure 1b). These progresses are due to both the use of high performance plant material (e.g., breeding for new cultivars with high fruit aesthetic quality and long shelf life; Brown and Maloney, 2003) and cultivation techniques through the improvement of the planning, establishment and management of orchards (Robinson, 2003).

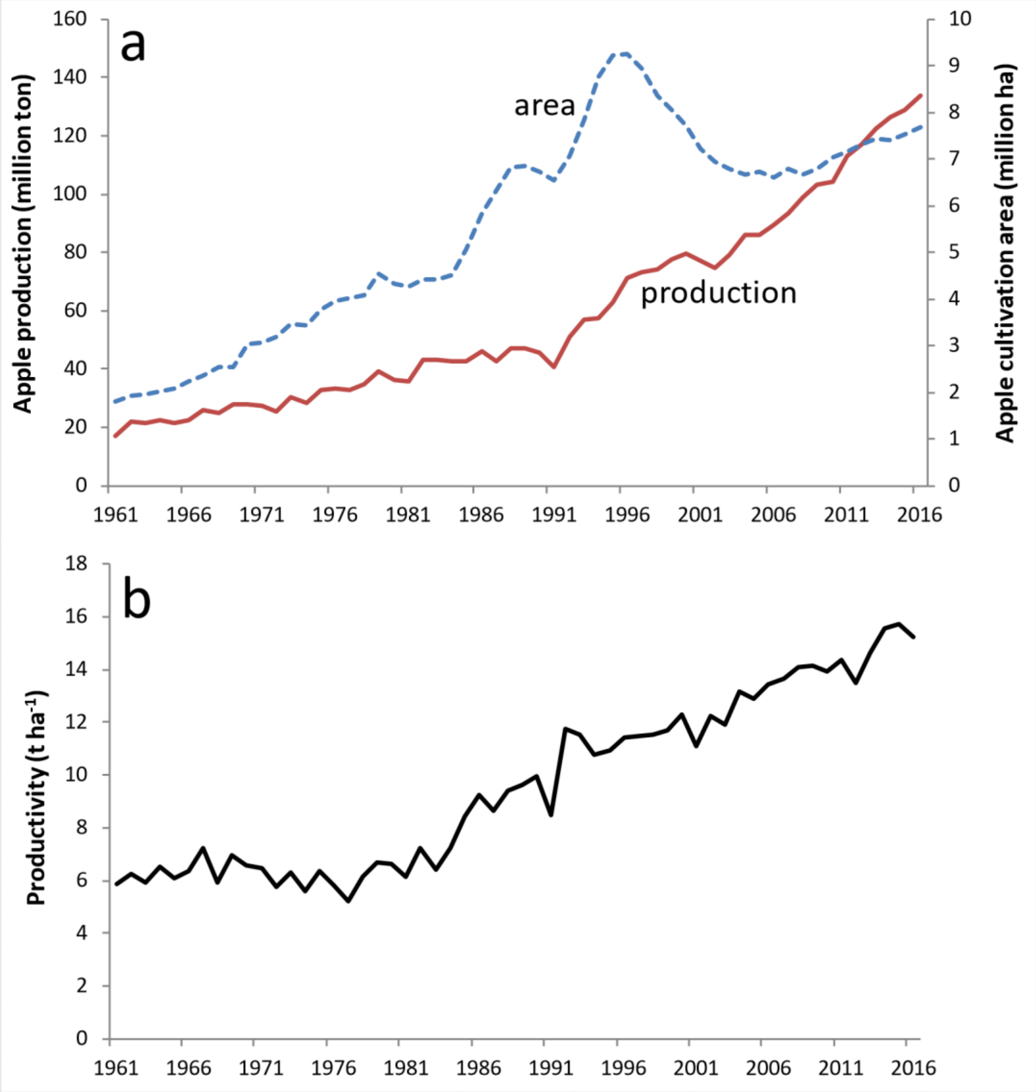

Figure 1. Progress in apple production (million ton) and cultivation area (million ha) (a), and apple productivity (t ha^{-1}) (b) in total world from 1961 to 2016 (FAO, http://www.fao.org/faostat/en/#data; for 'apple', 'all countries', 'all years', 'area harvested', 'production quantity' and 'yield'; accessed March 29, 2018).

However, this intensification of apple cultivation, often coupled with a 'weed-free' (or 'bare-soil') strategy eradicating all vegetation on the orchard floor, was done at the expense of an increasing reliance on pesticides, i.e., herbicides to limit competition for water and nutrients and phytosanitary treatments against pests and diseases, hereafter referred to as 'pests'. This reliance on chemicals with known adverse effects on the environment and human health is considered as the main obstacle to the sustainable intensification of agriculture in general (see for example in China; Lu et al., 2015). Apple remains one of the most treated fruit crops (EWG, 2017). Indeed, in order to achieve high yields of high quality fruit apple, orchards require frequent pesticide applications, up to ca. 35 according to a survey made in France in 2012 (MAAF, 2014) and more generally between 10 and 24 according to Granatstein and Peck (2017). Moreover, a strong reduction in the number of cultivars accompanied this intensification increasing the risk of narrowing the genetic ability to resist to or tolerate existing and new pests, and to adapt to climate change. For example, in Germany, it is estimated that the traditional multispecies 'streuobst' systems hosted a high apple genetic diversity with ca. 1400 cultivars which were robust and well-adapted to the local pedoclimatic conditions (Herzog, 1998) whereas in the current monocropping system 52% of apple production is covered by only five cultivars (Garming, 2013). A main conclusion is that current high density monoclonal orchards are hardly appropriate to drastically reduce pesticide use (Simon et al.,

2011) and that both the range of existing commercial apple cultivars and the design of the apple orchard system need to be reconsidered (Lauri and Simon, 2019).

In the following we review how initiatives such as 'integrated fruit production', 'organic farming', and 'agroecology' developed in past decades have renewed our way to establish and manage more sustainable systems of apple production. Secondly, taking inspiration from 'agroforestry' we show that apple-based systems may also be designed including other crops and considering a range of 'ecosystem services'. The need to foster fundamental and applied researches on how the apple tree interacts with its environment will be emphasized.

IMPROVING THE SUSTAINABILITY OF CONVENTIONAL ORCHARDS

Integrated fruit production, organic farming and agroecology

The idea to meet the global challenges of securing the food supply while reducing external inputs and minimizing negative impacts on the environment and human health were institutionalized in the 1980s (Granatstein and Peck, 2017). A common ground of the several initiatives developed in past decades is the concept of 'sustainability' which was defined as "the ability to make development sustainable to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland, 1987). In this general framework, three main apple farming systems have been developed in past decades that are still co-existing.

Integrated production (IP) was officially initiated in 1977 under the umbrella of IOBC (International Organisation for Biological and integrated Control) to promote environmentally safe methods of pest control, and later to promote the development and adoption of IP methods (Avilla and Riedl, 2003). Applied to fruit, integrated fruit production (IFP) is defined as "the economical production of high quality fruit, giving priority to ecologically safer methods, minimizing the undesirable side effects and use of agrochemicals, to enhance the safeguards to the environment and human health" (Malavolta and Cross, 2009). Continuous studies have been developed to improve IFP implementation, mainly about integrated pest management (IPM), including the use of biorational pesticides that express selectivity to specific developmental stages (e.g., biopesticides and insect growth regulators), semiochemicals (e.g., sex pheromones) and biological control (using natural enemies of pests) (Damos et al., 2015). In many countries, and also at the international scale through the impetus of institutions such as the Food and Agriculture Organization (FAO), conventional apple orchards have been encouraged to move towards IFP with the objective to minimize synthetic pesticide use (Damos et al., 2015). IFP can then be considered as the standard conventional apple orchard, at least in Europe where IPM is compulsory since 2009 (Damos et al., 2015).

Organic farming (OF) is defined as a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than on the use of inputs with adverse effects. OF typically puts emphasis on maintenance of soil organic matter via the use of organic composts, mulches and green manures (Neilsen and Neilsen, 2003) and also makes use of nitrogen fixing plants (Weibel and Häseli, 2003). In Europe, OF conforms to strict regulations that define precise farming and processing techniques (Migliorini and Wezel, 2017). A main difference between IFP and OF is that OF aims to manage the orchard system as a whole insisting on the ban of synthetic inputs and their derivatives (Weibel and Häseli, 2003). Organic farming combines "tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved" (IFOAM, 2005; Niggli et al., 2016). However, OF may also raise environmental issues such as those related to intensive use of copper in humid climates that can be harmful to plant growth and development, and to soil microorganisms and fauna (INRA, 2018), and that can increase soil compaction due to more machinery traffic (Nature's Path, 2016). Although the area of apple production in OF is strongly increasing since the past recent years (Granatstein et al., 2016), OF is still currently less developed than IFP with around 10% of the fruit-growing area in the main fruit production regions in Europe (Kienzle and Kelderer, 2017).

Agroecology (AE) combines knowledge in agronomy and ecology and has a defined set of principles for the ecological management of agrifood systems which extends what is developed in OF integrating the more general concept of 'ecosystem services' (see below) and especially socio-economic and political principles that are not or less explicitly managed in IFP and OF (Gómez et al., 2016; Migliorini and Wezel, 2017). Agroecology meets an increasing interest in apple production since it opens routes to design novel apple-based systems optimizing interactions between the apple tree and the other plants of the system (Simon et al., 2017). Although many cultivation practices are similar for IFP, OF and AE (e.g., choice of species and genotypes to optimize positive interactions and minimize negative interactions among them; management of soil structure and fertility; pest and weed management), the origin and quantity of products used and also often the design of the system, are different (Migliorini and Wezel, 2017).

To summarize, if the use of synthetic products differentiates IFP and OF, without a clear position of AE on this aspect, these latter two farming systems share a common holistic view towards improving the sustainability of the apple systems based on more knowledge on, and a better use of, interactions among plants, and between plants and the environment. Using the grid established by Hill (1998) to compare the various degrees of sustainability of agroecosystems, IFP would conform essentially to the 'efficiency' (i.e., improving the use of a given chemical input) and 'substitution' (i.e., replacing one input by another one or by another technique that is less disruptive) strategies. On the other hand, OF and even more AE are more in the 'redesign' strategy (e.g., with various spatial and temporal combinations of plant species around the apple) and management of the whole system at both agricultural practices and socio-economic levels. However, apart from the restriction on synthetic pesticides use specific to OF, practices (e.g., choice of associated plants, cultural operations) developed in IFP, OF and AE systems may have converged over time especially under the increasing constraints due to international or national regulations relative to the use of phytosanitary products.

Setting innovative apple orchards – example of initiatives reducing pesticide use

Simon et al. (2011) compared the level of use of fungicides and insecticides, and agrienvironmental performances of three farming systems, 'national IFP standards', in short 'IFP', 'low-input' (i.e., pesticide use as a last resort) and OF. The study also included the effect of the cultivar using three cultivars differing in scab susceptibility: 'Ariane' (Vf-resistant), 'Melrose' (low-susceptibility) and 'Golden Delicious' (susceptible). A significant farming systemcultivar interaction effect was found. Indeed, as hypothesized, the highest pesticide use was found for 'Golden Delicious' especially in IFP system. The least pesticide use (-43 to -56% compared to 'Golden Delicious') was found in 'Ariane' and 'Melrose' in both low-input and OF. However, considering yield and fruit damages, only 'low-input' 'Melrose' and 'low-input' 'Ariane' systems achieved similar results as the same cultivars in IFP. These results indicated that the choice of the cultivars according to their genetic resistance or tolerance to pests has a crucial role when implementing low pesticide systems.

Within a given farming system, the system design also plays a significant role in pest dissemination. For example, working on the apple-scab pathosystem without fungicides, and mixing a susceptible cultivar and a resistant cultivar in equal proportions, Didelot et al. (2007) observed a significant reduction in disease incidence over both study years (-7.3 to -21.3%), and severity in the second year (-35.4%) in the within-row mixtures, compared to the monoculture of the susceptible cultivar. Moreover, combining within-row mixture and a moderate fungicide treatment, disease incidence was reduced by 75.1% on leaves and by 69.7% on fruits. Going further and although not really applicable in practice, the modeling work developed by Sapoukhina et al. (2009) showed that random patterning of susceptible and resistant apple cultivars can reduce pathogen infection.

All together, these works indicate that, in a monospecies orchard, both the intrinsic tolerance or resistance of apple cultivars to pest and planting design can be combined to significantly reduce pest damages and/or at least permits reducing pesticide treatments. However, all these systems whether IFP, OF or AE are still dependent on regular and tightly adjusted inputs in terms of cultural operations and treatments. Improving apple cultivation sustainability needs revisiting actual concepts of apple cultivation, especially on two aspects that are developed in the following chapter, plant diversity 'around' the apple and plant arrangement.

REDESIGNING MORE DIVERSIFIED AND MULTIFUNCTIONAL APPLE-BASED SYSTEMS

The concept of ecosystem service

The Millennium Ecosystem Assessment (2005) and more recently the Common International Classification of Ecosystem Services (CICES; Haines-Young and Potschin, 2018) consider that a natural ecosystem or an anthropized ecosystem, i.e., an agroecosystem, can provide various ‘services’ defined as “the benefits people obtain from ecosystems” (Millennium Ecosystem Assessment, 2005). Typically, a conventional apple farming system aims at providing quite only a ‘provisioning service’, namely apple production. However, considering more complex systems such as OF and AE, there is a shift from a monofunctionality to a multifunctionality of the orchard. This multifunctionality may be handled using the ecological concept of ‘ecosystem services’ (Barot et al., 2017). These ecosystem services are grouped in three main categories: ‘provisioning’ (e.g., food, fibre, fuel), ‘regulation and maintenance’ (e.g., regulation of pests through the mixing of genotypes, nutrient cycling through the use of nitrogen-fixing grass in the alley) and ‘cultural’ (e.g., ‘pickyour own’ orchards, cultural and heritage values) (Haines-Young and Potschin, 2018). More precisely, Demestihas et al. (2017) identified five services that, apart from fruit production, can be provided to a satisfying level by an apple farming system: climate regulation, soil nitrogen availability, water regulation, pest and disease control, and pollination. These services can be optimized through the choice of plant material and agricultural practices. Both aspects can be optimized via the design of ‘agroecological infrastructures’ that include not only the management of the inter-row flower or grass strips but also the hedgerows composed of bushes and trees around the plot. The objective of these agroecological infrastructures is to host beneficial arthropods by providing food complements such as nectar, pollen and/or honeydew for optimal survival, fitness and fecundity (Simon et al., 2010, 2017; Albert et al., 2017; Demestihas et al., 2017).

Some studies have been developed on ecosystem services in apple orchards. Recently, Demestihas et al. (2018) modeled several ecosystem services (soil nitrogen availability, climate regulation, water cycle maintenance and regulation, and fruit production) at the annual scale and interactions between some of them. For example, considering apple production and denitrification, they showed that a weak crop reduces nitrogen needs and thus nitrogen absorption by the trees, therefore increasing nitrogen leaching.

Implementing ecosystem services in apple-based farming systems: combining plant diversity and plant arrangement

1. Increasing plant diversity.

Plant diversity is a main issue in OF and AE because it is the combination of the various plant species, whether annuals or perennials, grass, bush or even trees, that is the basis of ecosystem services provided by the system. Indeed, plant diversity is at the core of the three main classes of ecosystem services identified in all OF and AE systems (Migliorini and Wezel, 2017) and especially in apple-based OF and AE systems (Demestihas et al., 2017, 2018; Simon et al., 2017), namely, provisioning, regulation and cultural.

There is a consensus that functional differences among species are more important for biodiversity effects than species richness per se. This means that it is important to identify precisely the traits of the species (their ‘functional characteristics’) one wants to introduce in the system and the way they can be managed, e.g., soil cover, nitrogen fixing, host for natural enemies (Bakker et al., 2018). Several species can share a given functional trait and in this case the choice of only one of these species may be sufficient. For example, considering soil nitrogen availability, biological nitrogen fixation can be provided using various legumes such as white clover (*Trifolium repens*) but also alfalfa (*Medicago sativa*) or sainfoin (*Onobrychis viciifolia*) and the choice of one of those species depends more on the will to grow an annual or a perennial grass cover in relation to technical and economic considerations, and pedoclimatic conditions. However, some redundancy may be useful to face disruptions caused by climatic events (Duru et al., 2015). For example, pest control often relies on a range of resources in the agroecosystem, e.g., plant assemblages providing natural enemies with resources and habitat all year long (Simon et al., 2010) or ‘banker plants’ that serve as alternative hosts for a parasitoid or predator of the target crop pest (Demestihas et al., 2017). Plant diversity also needs to be considered in interaction with management even though a given practice can have various effects on pest dissemination depending on the period of the year, and above all the presence of alternative resources. For example, codling moth sentinel egg predation in apple canopy mainly due to the earwig *Forficula pubescens* is significantly higher in alleys with tall grass than in frequently mowed alleys maintaining short grass in June, but the opposite was observed in July when alternative food was available in the tall grass (Marliac et al., 2015).

Apart from the provisioning and the regulation services, plant diversity in the applebased system also provides cultural services, consisting for example in the heritage value of some endemic or cultivated plants grown in the orchard and its surroundings, or from another point of view the enjoyment of 'pick your own' activities for families, not only of apple but also of other fruits or aromatic plants, especially around large cities.

2. Plant arrangement: insights into the structural and temporal dimensions.

Plant diversity needs to be considered along with the spatial arrangement, and the within-time and within-space interactions have to be considered in the design and the decisional system (Simon et al., 2017). In typical OF and AE where apple production is the main production, apple trees are planted in rows and the agroecological infrastructures and agricultural practices are mainly devoted to pest regulation (e.g., Albert et al., 2017) and soil nitrogen availability (Demestihas et al., 2017). However, apple trees can also be grown with plants providing other production such as soft-fruits, vegetables and aromatic plants that can be combined with plants providing pest control and/or soil nitrogen availability services (e.g., 'verger maraîcher' in France, GRAB, 2017; 'silvoarable agroforestry' in UK, Smith et al., 2016). Moreover, such diversity also enables to design 'pest suppressive' agroecosystems (i.e., highly unfavourable to pests and highly favourable to their natural enemies) through barrier-dilution effects, push-pull processes with trap and repulsive plants, and avoidance strategies beside conservation biocontrol.

In typical OF and AE, apple-trees are in most cases at the higher stratum, i.e., in full sun whereas all the other plants, as bush or grass, occupy the lower strata below apple trees. However, more complex systems may combine plants in various strata: large trees (e.g., nut and timber trees) over-topping the apple, with shrubs (e.g., black currant) and possibly annuals (e.g., corn-soybean rotation) in the understory. These systems are identified as 'multifunctional woody polycultures' (Lovell et al., 2018). They resemble traditional 'agrisylvicultural systems', and also 'agrosilvopastoral systems' (Parrotta et al., 2015) when they combine grazing sheeps or chickens that can eat fallen leaves possibly infected by scab and pest arthropod larvae on the ground (Burgess et al., 2017; Corroyer and Upson, 2015; McAdam and Ward, 2018). These systems belong to 'agroforestry' that is a contraction of the terms "agriculture and forestry and designates land use that combines aspects of both, including the agricultural use of trees" (van Noordwijk et al., 2016). Fruit trees are the primary driver of agroforestry adoption worldwide especially in the tropics (Wolz and DeLucia, 2018) and are considered as high value for agroforestry (den Herder et al., 2017; Pantera et al., 2018; Lauri et al., 2019).

Agroforestry and agroecology are two approaches of complex multifunctional systems, with agroforestry including explicitly woody plants. It is worth noticing that agroforestry is not mentioned in the European regulations and IFOAM (2005) norms concerning crop production practices in OF and AE (Migliorini and Wezel, 2017). However, it is considered that agroforestry offers a relevant framework for agroecological practices (Wezel et al., 2014). Although agroforestry considers mainly the structural and temporal arrangement of the system associated to different uses (e.g., in the USA: alley cropping, silvopasture, riparian buffers, windbreaks and forest farming; Wilson and Lovell, 2016), agroecology clearly addresses the ecological-driven functioning of the system (Wezel et al., 2014). It should also be mentioned that agroforestry systems have no guidelines about pesticide use even though those systems are "less reliant… on the use of synthetic pesticides" compared to simplified and specialized cropping systems (UCDAVIS Agricultural Sustainability Institute, 2018) (Figure 2).

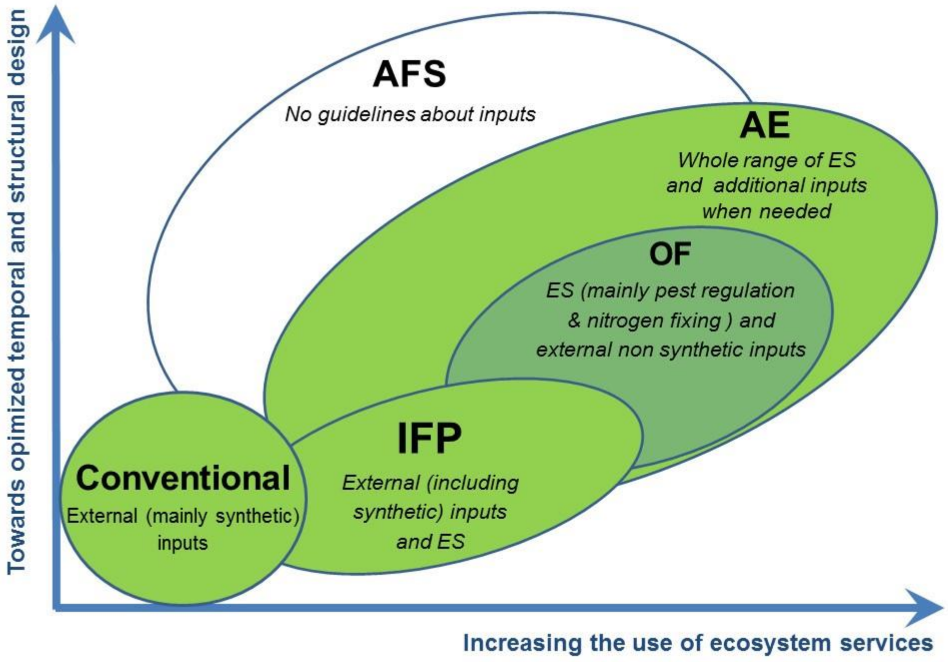

Figure 2. Sorting apple agroecosystems according to the use of ecosystem services (ES) and the optimization of temporal and structural design. The horizontal axis renders, from left to right, the progressive use of ES. The conventional orchard typically relies on the use of external (mainly synthetic) inputs whereas integrated fruit production (IFP) aims to efficiently manage those synthetic inputs with the possible use of some ES and alternative methods. Organic farming (OF) includes the use of some ES, mainly natural pest regulation and nitrogen fixing by plants, and no use of external synthetic inputs. Agroecology (AE) manages the whole range of ES (see text) with a better emphasis on societal needs. OF may be considered as fully included in AE. The vertical axis renders, from bottom to top, the improvement of the structural and temporal design of the system taking advantage of concepts developed in agroforestry. There are no guidelines about inputs in agroforestry systems (AFS).

Designing 'apple-tree based agroforestry systems' (AT-AFS) that would include the apple in a multistrata system complementing apple production with other productions (other fruits, vegetables, aromatic plants; animals) and providing other services such as those already included in OF and AE, is challenging. Some indications on the spatial arrangement of such complex systems already exist in literature. For example, although OF and AE may keep on planting distances usual for IFP, i.e., 3.5 m to 6 m between tree rows and 1 to 4 m between trees within the row, depending on the cultivar-rootstock combination (Smith et al., 2016), 'multifunctional woody polycultures' have larger distances with ca. 9 m and ca. 4 m between rows and within the row, respectively (Lovell et al., 2018).

3. Potential advantages of apple-tree based agroforestry systems.

A main economic interest of OF, AE and further AT-AFS would be to optimize the global productivity per land area but it has to consider the fact that not all productions are at the same pace. For example in an AT-AFS that would combine apple and timber trees, apple production is at the annual pace whereas timber is only valorized after 20 to 50 years. Such improvement in area productivity is all the more true if there is deep and rich soil and complementarity in vegetation cycle. This is typically the case for an agroforestry system combining winter wheat harvested at the end of spring and walnut with late budburst thus with a little overlapping in the growing cycles. Various metrics exist to evaluate the performance of multiproduction systems, among which the land equivalent ratio (LER) is the most known (Lovell et al., 2018). LER compares yields of crops grown together to crops grown as pure stands. For example for a system with two species: LER = (mixed yield A/pure yield A) + (mixed yield B/pure yield B).

Although, to the best of our knowledge no statistics exist for AT-AFS, the LER of a cherrywalnut-annual intercrop (sunflower, durum wheat, barley, oilseed rape) agroforestry system is close to 1.3, i.e., 1 ha of cherry-walnut-annual intercrop agroforestry may produce as much 1.3 ha of cherry, walnut and annual intercrop in separated stands due to fact that cherry and annual intercrop grow in the inter-row of walnut that is otherwise not used, even if in the combined system the inter-row of walnut is a little larger than in pure walnut stand (Dupraz et al., 2005). Beyond this global economic interest that can be valorized only in the long term, depending on the species production, AT-AFS should also ensure more stable economic returns over the years because it is based on various plant productions (e.g., vegetables; GRAB, 2017) and possibly animals such as poultry or sheep (see above) provided the apple tree shape is redesigned or the presence of sheep in the orchard is restricted from fall to spring before trees begin to grow (Lauri et al., 2019).

GOING FORWARD: RESEARCH ROUTES FOR THE FUTURE

Most of our knowledge in apple tree architecture and physiology has been developed removing as much as possible influences of the global biotic and abiotic environment. When external variables (temperature, irrigation, nutrition, etc.) are considered in these studies they are generally well focused and research seldom considers combination of variables for obvious reasons of complexity. These research works are well-adapted to the setting of conventional apple orchards whose performance is tightly related to the good integration of all the components of the 'orchard system puzzle', namely the plant material (rootstock, tree quality), the system design (tree arrangement and density, support system) and cultural operations (tree training and pruning, irrigation, fertilization) (Barritt, 1992).

Designing apple OF, AE or AT-AFS, needs more research on both plant-plant and plantenvironment (biotic and abiotic) interactions. The ecological science already provides some main concepts, such as the 'competition-facilitation' framework (e.g., interactions between trees or shrubs and grass; Scholes and Archer, 1997) that now need to be better applied in the field of apple diversified systems. From the 'plant science' point of view we need more knowledge on the plasticity of plant architecture and ecophysiology in response to the more complex biotic and abiotic environment.

Belowground niche partitioning

The interest of multispecies systems was already promoted by Gliessman (1985) considering that although multiple cropping systems "use more water, they are able to obtain water not available to monoculture", and therefore "they use the water more efficiently, and contribute significantly to soil conservation". These systems including ground covers between rows also reduce runoff (Demestihas et al., 2017) and soil compaction (Merwin, 2003). These multispecies systems therefore demonstrate the further potential for their more widespread use. More generally, interactions at the belowground level need more detailed studies. The positive effects of legumes as groundcover in the inter-row alleys (e.g., white and red clover, *Trifolium repens* L. and *Trifolium pratense* L., respectively, or alfalfa, *Medicago sativa* L.) are well known for their contribution to soil nitrogen (Merwin, 2003). This typically illustrates the facilitation process where the root systems of the various species explore a same soil layer. However, several lines of evidence would show that an aboveground multistrata system is also mirrored, even to a lesser extent, by multilayer root systems with annuals being mostly in the top soil and trees having deeper rooting. For example, in an experiment on walnut-winter cereals, Cardinael et al. (2015) show that competition induces deeper rooting of the trees keeping the perennial grass or annuals in the top soil horizon. Nevertheless, most of these studies are realized without irrigation supply in either wetlands or drylands. In these conditions, hydraulic redistribution (HR) has been measured from moist to drier portions of the soil (Domec et al., 2010) but also from soil to roots and from roots to soil and among overstory trees and understory shrubs and grasses (Barron-Gafford et al., 2017). To what extent HR is maintained in the context of an irrigated apple system needs to be reconsidered. First, root distribution is highly opportunistic being concentrated in the wetted zones determined by the irrigation type and scheduling (Sokalska et al., 2009) raising the question of the compatibility between localized irrigation and the maintenance of root layering. Second, root distribution in the soil is dynamic depending on tree and grass age (Scholes and Archer, 1997) possibly related to changes in tree root architecture between the young and the mature stages of the tree (e.g., in peach, secondary vertical roots do not develop before the second year in a peach-grass system with regulated deficit irrigation; Forey et al., 2017). According to Demestihas et al. (2017) working on an apple mixed with cover crops orchard, such optimal belowground niche partitioning could be a way to decrease the loss of water and also nutrient leaching to the water table.

Growing apple in the shade of over-topping trees

The possibility to grow apple trees with other, possibly over-topping, trees remains to be explored. In hot and dry summer climate, typically in the Mediterranean type climate, apple trees may be subjected to an excess of solar radiation during the summer months with known deleterious effects on fruit quality (sunburn) and leaf functioning (photooxydative damage) (Correlli-Grappadelli and Lakso, 2007; Racsko and Schrader, 2012). This issue is managed using shade nets, also used as hail nets, that reduce light quantity and may also change light quality with known effects on fruit colouring (Mupambi et al., 2018). To what extent 'shade trees' may be used instead of shade nets to mitigate climate extremes (heat, light, precipitation) effects on apple, especially in the context of climate change, remains to be studied especially regarding both distances between overstory trees and apple trees and the proper pruning of the overstory trees to ensure that sufficient light reaches the apple trees. Further research in apple may take lessons from the increasing amount of works developed in the tropics on two fruit trees, cocoa and coffee, known as shade-adapted species and that are traditionally cultivated in multistrata systems. Research on architecture and ecophysiology (Tscharntke et al., 2011; Padovan et al., 2018) and plant-pest interactions (Andres et al., 2016) in cocoa and coffee also documents the agronomic and economic issues related to fruit-tree growing in agroforestry systems. Preliminary results obtained in our experimental AT-AFS at INRA Montpellier ('GAFA' project; Pitchers et al., 2020) indicate that apples trees planted at 6.5 m from walnut trees beneficiate from a gap fraction (fraction of view that is unobstructed by walnut canopy, leaves and branches, in any particular direction) of ca. 70%. This value is close to the reduced photosynthetic active radiation usually measured under hail nets, i.e., 32.8%, without altering air temperature, humidity or rainfall, and slightly increasing yield (e.g., apple; Bosco et al., 2018).

Adapting apple plant material and optimizing training and pruning

Once the whole system is designed, including structural (spatial arrangements in horizontal and vertical dimensions) and temporal aspects (e.g., simultaneous vs. delayed plantation of the various species), the choice of the rootstock and cultivar and the training and pruning of the apple trees are crucial. Semi-vigorous or high-vigor rootstocks from the Malling Merton series (Ferree, 1988) or from the Cornell-Geneva series (Lordan et al., 2017; Fazio, 2017) for example, should be recommended to improve anchoring in the soil, and nutrients and water absorption in a context of above and belowground competition. This choice also needs to consider the resistance or tolerance to some major pests and diseases such as fire blight caused by *Erwinia amylovora*, crown and root rot caused by *Phytophthora cactorum*, and also replant disease complexes (Fazio, 2017). The use of own-rooted trees with high vigor (Maguylo and Lauri, 2007) could also be evaluated in these contexts. After plantation, training and pruning procedures also need to be well adapted to a reduced light climate. Research developed in past decades in tree architecture management clearly indicates the importance of increasing canopy porosity to air and light through precision pruning, for example using 'artificial extinction' (Lauri et al., 2004; Willaume et al., 2004; Lauri and Corelli-Grappadelli, 2014) with beneficial effects on fruit size and return-bloom (Lauri et al., 2007; Tustin et al., 2012; Breen et al., 2014, 2015, 2016; Tustin and van Hooijdonk, 2016) and partly on pest reduction (Simon et al., 2006). However, overstory canopy also needs to be managed and partial pollarding of trees can both provide fodder, fuelwood or woodchips as a provisioning service, and improve light transmission to the understory especially the apple tree as a regulation service (Le Bec et al., 2016).

Developing links with socio-economics to optimize the market chain

Eventually, the socio-economic impact of such diversified systems also needs to be assessed. As mentioned by Smith et al. (2016) in concluding remarks on two apple case studies, agroforestry seemed to have benefits in terms of reducing pest levels if apple cultivars are resistant or tolerant to major pests. It "could work well in a diverse, potentially small-scale system such as a market garden, where apples could contribute to direct marketing channels such as vegetable box schemes or farm shops" (Smith et al., 2016). Those agroforestry systems are recent, and existing and up-coming systems are to be considered and studied as well as the agrifood system they participate in (GRAB, 2017).

CONCLUSIONS

As for the 'industrial agriculture' in general (Struik and Kuyper, 2017) conventional apple orchard, characterized by high level of regular inputs (water, nutrients, pesticides, mechanization), is likely to remain the dominant source of apple supply as far as external resources are kept at a rather low price and negative externalities on the environment and human health are not considered. However, such a production system cannot be sustainable in the long term and/or in all production regions in the world especially due to its dependence on non-renewable energy and also its negative long-term impacts on human health and environment (Brundtland, 1987). We consider that moving towards more self-regulating, environment-friendly apple production systems, as already engaged in the last decades with IFP, OF and more recently AE, offers promising opportunities. We propose that managing more efficiently the structural and temporal design of these complex systems under the paradigm of AT-AFS is another step in the same dynamics (Figure 2). Here we emphasize the interest to diversify short-term and long-term productions of apple and other products, to design more 'pest suppressive' and 'nitrogen-autonomous' agroecosystem and also to better explore the climate mitigation offered by the multistrata design of the system.

Implementing such systems needs more knowledge from the analytical side on how plants interact among them and with the environment with regard to access to above- and belowground resources. It also requires more interdisciplinary approaches gathering horticultural science (e.g., yield performance and fruit quality of apple in reduced light climate) and ecology including chemical ecology (e.g., volatile organic compounds, VOC) and landscape ecology (e.g., effects of landscape heterogeneity on pests dissemination). So far, we lack references on complex apple-based agroecosystems, because traditional systems have almost disappeared except in few sites (e.g., cider apple orchards in Brittany, France). We make a plea for more participatory research involving growers, technicians, researchers and stakeholders in the market chain to strengthen the "economic, biodiversity, resource protection and social values of orchards" (Robertson et al., 2012) with practical outputs combining scientific and empirical knowledge.

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ABSTRACT

Agroforestry systems structured around fruit trees to produce fresh fruit is still under-developed in temperate zones. This study is based on the idea that the fruit tree can be integrated into multi-strata agroforestry systems where it would be grown with timber trees occupying the upper stratum and shrubs and/or herbaceous plants in the lower stratum. In addition to the production of fresh fruit, such systems would then combine different agro-ecosystemic services. The study focuses on a major temperate fruit species at the national and global levels, the apple tree. The general objective is to acquire a detailed knowledge of the tree's architectural development, its flowering and the quality of its fruiting, along these competition gradients. The work focuses on three actions: (i) defining an indicator to characterize each apple tree environment in this complex agrosystem, (ii) analyse at the tree scale the impact of agroforestry on morphological, phenological and architectural traits, and (iii) analysing the daily and annual sap flow regarding environmental variables and in relation to the aforementioned architectural traits. Using the light as a variable to analyse our architectural data, we have shown that apple trees did express shade avoidance traits affecting morphology (decreased taper and increased slenderness and specific leaf area), architecture (fewer growing shoots and proportion of flower clusters) and phenology (reduced number of days at full bloom). Finally, we have shown that sap flow and transpiration per unit of leaf area was affected by environmental variables (vapour pressure deficit and reference evapotranspiration). Shade did not change apple trees sap flow daily dynamics and reduced water and transpiration per unit of leaf area mainly because of morphological and architectural adaptation to shade in our experimental conditions. An increase of leaf area or a complexification of the apple tree architecture (i.e. the number of ramifications) increased transpiration per unit of leaf area during the summer. Our results suggest that while the architecture of apple trees is modified by a reduction in light intensity, it is not until a reduction of 65% that the capability to produce fruit is impeded.

RESUME

Les systèmes agroforestiers structurés autour des arbres fruitiers pour la production de fruits frais sont encore peu développés dans les zones tempérées. Cette étude est basée sur l'idée que l'arbre fruitier peut être intégré dans des systèmes agroforestiers multi-strates où il serait cultivé avec des arbres à bois occupant la strate supérieure et des arbustes et/ou des plantes herbacées dans la strate inférieure. Outre la production de fruits frais, ces systèmes combineraient alors différents services agro-écosystémiques. L'étude se concentre sur une espèce fruitière tempérée majeure aux niveaux national et mondial, le pommier. L'objectif général est d'acquérir une connaissance détaillée de l'établissement architectural de l'arbre, de sa floraison et de sa nouaison, le long de ces gradients de compétition. Le travail se concentre sur trois actions : (i) définir un indicateur pour caractériser l'environnement du pommier dans cet agrosystème complexe, (ii) analyser à l'échelle de l'arbre l'impact de l'agroforesterie sur les caractéristiques morphologiques, phénologiques et architecturales, et (iii) analyser la dynamique de flux de sève journalière et le cumul annuel en fonction des variables environnementales et en relation avec les caractéristiques architecturales susmentionnées. En utilisant la quantité de lumière comme variable pour analyser nos données architecturales, nous avons montré que les pommiers exprimaient des traits d'adaptation à l'ombre affectant la morphologie (diminution de la conicité et augmentation de l'élancement et la surface foliaire spécifique), l'architecture (moins de pousses en croissance et d'inflorescences) et la phénologie (réduction de la pèriode de pleine floraison). Enfin, nous avons montré que la densité de flux de sève n'était pas affecté par nos variaibles envionnementales (déficit de pression de vapeur et évapotranspiration de référence) contrairement à la transpiration par unité de surface foliaire. Dans nos conditions expérimentales, l'ombre n'a pas modifié la dynamique quotidienne du flux de sève des pommiers et a réduit la transpiration par unité de surface foliaire, principalement en raison d'adaptation morphologique et architecturale. Une augmentation de la surface foliaire ou une complexification de l'architecture du pommier (c'est-à-dire le nombre de ramifications) a augmenté la transpiration par unité de surface foliaire pendant l'été. Nos résultats suggèrent que si l'architecture des pommiers est modifiée par une réduction de l'intensité lumineuse, ce n'est qu'à partir d'une réduction de 65% que la capacité à produire des fruits est entravée.