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# ORAL PRESENTATIONS



# Kiwifruit vine decline syndrome and other emerging and re-emerging biosecurity challenges

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Climate change and market globalization are posing new threats to crop protection by facilitating pathogens and pests spread and establishments in new areas. Moreover, climate change is causing a constant increase in the frequency and severity of plant stresses especially during delicate phenological phases such as blooming, fruit set and pre-harvest. In climate change hot-spots, such as the Mediterranean Basin, the increase biotic and abiotic stresses related to the forecasted climate change scenarios are predicted to lead to a significant decrease in crop yield, paralleled with a higher inter-annual variability. From 2012, in Europe 142 new pathogen introductions were recorded including viruses (37%), bacteria (32%) and fungi (25%). This situation is further worsened by the 50% reduction of pesticide use and risk foreseen by 2030 by the European Green Deal. Kiwifruit is no exception and, in the last decade, new pests (*Halyomorpha halys*), pathogens (*Pseudomonas syringae* pv. *actinidiae*, *Phytophthora* and *Pythium* spp.) and physiological disorders [kiwifruit vine decline syndrome (KVDS), pre-harvest fruit drop] have emerged. The latter phenomena, being complex idiopathic syndromes, are particularly difficult to control. Among these emerging syndromes, KVDS is the most important causing a severe and rapid decline that leads to vine collapse within two years from symptom development. KVDS affects both *Actinidia chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* and is prevalent in soils affected by waterlogging or poor aeration, suggesting a physiological contribution to this disorder. Symptoms generally become more evident in conditions of high physiological activity and evapotranspiration. Currently, the only control options are mitigation strategies, which, however, are applied when symptoms develop, and, thus, have a limited effect in preventing yield and quality losses. In experimental conditions, imposed waterlogging in conditions of high evapotranspiration is not sufficient, per se, to cause KVDS symptoms. Recent evidence suggested a role of the rhizosphere microbial community, since healthy and KVDS-affected plants show distinct bacterial and fungal communities. These changes are creating a disbiotic condition leading to KVDS on plants with a chronically stressed root system. Different pathogenic microorganisms, such as *Phytophthora* spp., *Phytophthora vexans* and *P. chamaeophos* spp., *Desarmillaria tabescens* and *Clostridium bifermentans* and *C. subterminale*, have been found associated to these changes in the rhizosphere microbiome. Despite each of the pathogen being able to induce, in artificial conditions, severe symptoms resembling KVDS, the complex interplay between the changes in the root environment and KVDS onset still needs further studies to be elucidated. Several research programs are aiming at dissecting the complex mechanisms underlying KVDS development and pilot projects have been initiated to develop short- and medium-term mitigation measures. These include precise irrigation tailored on plant needs, soil amendment and use of biological control agents. However, effective mitigation strategies should rely on the precise deciphering of the functional changes occurring in the rhizosphere microbiome, the signalling network governing these interactions and the physiological and molecular response of the vine. Indeed, these studies will allow to develop tools for an early detection of KVDS which is of paramount importance to develop timely intervention strategies prior symptom occurrence. Finally, long term strategies for minimizing these problems and mitigating climate change effects should include breeding for new rootstock and varieties, the adoption of novel semi-protected cultivation methods, the use of sensitive and stress-specific method for precision agriculture and the training of growers and technicians.

# Analysis of global kiwifruit research and application progress based on SCI journal papers

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Based on the Web of Science, this study focused on 2716 kiwifruit research papers included in SCI and CNKI core journals from 2003 to 2022. Bibliometric analysis was used to examine the publications by years, countries or regions, and subject areas. Results indicated a general increasing trend in the number of SCI over the last 20 years. China was the country with the highest number of SCI papers, followed by New Zealand and Italy. The institutions with the highest number of papers were Northwest Agriculture and Forestry University, Wuhan Botanical Garden of Chinese Academy of Sciences, and Jiangxi Agricultural University. Kiwifruit research involves 131 disciplines including plant science, horticulture, plant pathology and entomology, fruit storage and processing, molecular biology, biochemistry, pharmacology, and bioinformatics, with interdisciplinary research papers. The fastest growing disciplines in the past 5 years are molecular biology, plant pathology, and fruit storage. Analysis of journal papers provides information and reference for researchers to understand global kiwifruit research dynamics, establish research directions, and conduct academic exchanges.

# Impact case study of kiwifruit maturity research for the New Zealand industry

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Over the last 25 years, the volume of kiwifruit exported from New Zealand has approximately trebled, with a concomitant increase in value. The industry has also increased in complexity with the introduction of yellow- and red-fleshed *Actinidia chinensis* var. *chinensis* fruit, in addition to the traditional, green-fleshed *Actinidia chinensis* var. *deliciosa* 'Hayward' fruit. The increased volumes and cultivar complexity provide challenges for the industry. Extending the harvest window ensures New Zealand-grown kiwifruit are available to consumers for longer, whilst helping packhouses, cool stores and exporters better manage their labour and logistics. Fruit is available in key markets earlier, securing shelf space and premium prices for early season sales, and growers receive an incentive payment for meeting the requirements of the early season KiwiStart programme. Likewise, later harvested fruit provide supply after prolonged storage, extending the supply window at the end of the season. KiwiStart was originally developed for 'Hayward' fruit in the late 1990s. The system incorporates a range of indicators to ensure that fruit meet the industry's quality standards, based on knowledge of kiwifruit maturation, storage, ripening and consumer acceptance characteristics. The indicators are specific to each cultivar, and, with the more recent introduction of new yellow-fleshed and red-fleshed cultivars, additional research has been required to understand and manage the de-greening of the fruit, ensuring customers receive kiwifruit with consistently vibrant coloured flesh.

## Sensory research of kiwifruit: a journey

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Delivering kiwifruit with highly preferred consumer-sensory properties is critical to the success of this fruit category. The extent to which sensory properties meet or exceed consumer expectations is influenced by genetics, environment, and orchard management practices, and modified by both postharvest storage conditions and the way consumers handle fruit in their homes. The following framework and definitions are useful when conceptualising consumer-sensory perceptions: (1) eating quality n all those characteristics of a food that lead a consumer to be satisfied with the eating experience; (2) texture n the sensory manifestation of the structure of the food; (3) flavour n the impressions perceived via the chemical senses from a product in the mouth. All these aspects of kiwifruit fundamentally rely on human perception as the benchmark measure. In this presentation we give an introduction into recent advances in understanding the importance of biological determinants of eating-quality in kiwifruit, the advances in the methodologies used to characterise sensory properties, and approaches to conceptualise the potential impacts of different sensory properties.

# A new method to assess early kiwifruit decline with Sentinel-2A satellite images

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Kiwifruit decline (KD) evolution in time can be very fast. In extreme cases, it can lead to the complete decline of an orchard within a few weeks. Monitoring KD in orchards requires significant human, financial and time resources. Since 2017, Sentinel-2 satellites have been providing aerial images every 5 days. Their 13 spectral bands are used to calculate vegetation indices (VI), enabling plant biophysical properties to be monitored and giving an indication of plant biomass. We hypothesize that the normalized difference vegetation index (NDVI) is a good indicator of kiwifruit decline. We studied 3 orchards located in the South-West of France with different intensities of decline. The Marmande orchard presented contrasting situations, with areas of high decline intensity. In May and September, a strong negative correlation between pixel-average vine health and NDVI was found ( $r=-0.90$ ,  $p$  value  $< 0.001$ ). For the Sorde-l'Abbaye orchard, the correlation was high for September ( $r=-0.51$ ,  $p$  value  $< 0.001$ ) but not significant in May ( $r=-0.07$ ,  $p$  value  $> 0.05$ ). In the Moissac orchard, dead, absent or declining trees are sparsely distributed across the plot, so there was no correlation between NDVI and percentage of decline ( $r=-0.24$ ,  $p$  value  $> 0.05$ ). In the short term, NDVI could be used as an early signal to follow KD progression in space and in time at the orchard scale in the case of a spotty progression of KD in orchards, but in the case of sparse progression in an orchard, image analysis with finer resolution than Sentinel-2 images should be carried out.

## A new systemic approach for promoting soil and plant health in G3 orchards to face kiwifruit vine decline syndrome (KVDS)

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Italy, the third largest producer of kiwifruit in the world, lost 10% of its production in recent years because of the spread of the kiwifruit vine decline syndrome (KVDS). Although the causes of KVDS are still not clear, it is often associated with water excess and stagnation. We hypothesize that soil compaction or soil structure disruption associated with hypoxia or hyper oxidation, could have a priming effect in the emergence of KVDS. To investigate the causal factors and potential solutions to counter KVDS, a multi-disciplinary experimental trial was undertaken in a kiwifruit orchard (*Actinidia chinensis* var. *chinensis* 'Zesy002') affected by KVDS in Latina (central Italy). After a first phase of fundamental agronomical ameliorations, such as water drainage and precision irrigation (very relevant to lower the water table), nature-based solutions were applied, such as sowing decompacting crops for increasing water permeability, and the application of compost and bio-fertilizers containing plant-growth-promoting microorganisms. Also, following a deep study on the root physiology in KVDS vines, a root pruning approach was tested for improving the regeneration of roots, whose status was seriously compromised by KVDS. The adoption of all these sustainable agronomic practices ameliorated the general physiological status of the plants (+46.1% of healthy plants), improved soil characteristics (e.g., +5% macroporosity, shifts in redox potential, and lower water table), and determined changes in rhizosphere microbiota composition and functioning. The results demonstrated that a sustainable and agroecological approach to kiwifruit orchard management can represent a technically feasible solution to halt the spread of KVDS by recreating the natural environmental conditions where kiwifruit plants thrive. We are confident that, for facing KVDS, it should be adopted a systemic approach that considers plants as an integrated system with soil and atmosphere, where rhizospheric and endophytic microbial communities play a key role in modulating and translating environmental factors in plants, that have to be considered as "holobionts". This "one-health" approach highlights that the physiological status of kiwifruit plants is not isolated but connected to the health of the other components of the agroecosystem, in particular with soil and potential plant-growth-promoting microorganisms living in it or inhabiting plant tissues.

## **Phytophthora biodiversity in New Zealand kiwifruit**

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Kiwifruit vine decline syndrome (KVDS) is a serious problem in kiwifruit industry especially in Italy. The aetiology of KVDS is uncertain, with soil waterlogging considered an important factor, and a wide variety of microorganisms associated with symptomatic plants. Water logging conditions are favourable to the species of *Phytophthora*. A review into the potential impact of *Phytophthora* species to New Zealand kiwifruit recommended that New Zealand should be in a "state of alert for *Phytophthora* attacks on kiwifruit" and "determine the risk of *Phytophthora* species that are present in New Zealand" (Woodward & Boa 2019). In response, Kiwifruit Vine Health (KVH) and Zespri Group Limited, The New Zealand Institute for Plant and Food Research Limited (PFR) investigated the biodiversity of *Phytophthora* species across different regions where kiwifruit are grown in New Zealand and over different seasons and years, and determined the relative risk of *Phytophthora* species on New Zealand kiwifruit vines. Samples were collected from 128 sites in 2020 to 2022. At each sampling event, four vines per site were sampled, mostly from *Actinidia chinensis* var. *deliciosa* 'Hayward' and/or *A. chinensis* var. *chinensis* 'Zesy002'. Soil, leaf, and root segment samples were collected from each vine and analysed by either baiting (soil and some leaf samples), immunostrip (leaf samples) or by using eDNA techniques (root segment samples). No *Phytophthora* species were detected in the leaf samples from the 128 sites using baiting and/or the immunostrip assays. *Phytophthora* species were putatively identified by baiting are *P. cactorum*, *P. chlamydospora*, *P. cinnamomi*, *P. citricola/plurivora*, *P. citrophthora*, *P. cryptogea*, *P. gregata*, *P. medicaginis* x *cryptogea* -like, *P. megasperma*, *P. multivora*, *P. pseudocryptogea*, *P. acerina* -like and *P. syringae* which are already present in New Zealand. Therefore, the current risk of *Phytophthora* disease in kiwifruit orchards remains low in New Zealand. This survey establishes a baseline for changes in *Phytophthora* populations in New Zealand orchards, such as those that might be associated with vine decline. This study also provides a basis for robustly identifying future changes in species diversity in New Zealand kiwifruit orchards and biosecurity.



## Evolutionary paths of a Japanese Islands-specific various polyploidy in kiwiberry (*Actinidia arguta*)

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In plants, polyploidization potentially contributes to the acquisition of novel beneficial traits via adaptation to a new environmental condition, often involved in historical domestication events or recent crop breeding. Hardy kiwi (*Actinidia arguta*) is supposed to be basically tetraploid (or hexaploid), whereas this species has evolved various polyploid populations ranged from diploids to octaploids, specifically in the Japanese Islands. Importantly, they exhibit transitions of some beneficial traits, including expression of parthenocarpy, in highly ploidy-dependent manners. In this study, we attempted to uncover the evolutionary process to form these various polyploid populations and the mechanism for ploidy-dependent parthenocarpy expression, based on whole-genome wide resequencing data. Combinations of population structure analyses using genome-wide 99,736 SNPs of 74 hardy kiwi lineages (diploid to octaploid) suggested that these 2-8X polyploidy populations were not established via a ploidy-dependent unidirectional process but via complex interploidy hybridizations. The tetraploid population with the widest geographic distribution and the highest genetic diversity formed three subpopulations, of which each was derived from two distinct diploid populations (from eastern and western areas in Japan) and their admixture, respectively. The heptaploid and octaploid populations were suggested to be derived from interploidy hybridizations of tetraploid and hexaploid subpopulations with fundamentally distinct genome structures. Thus, they would convergently evolve ploidy-dependent phenotypic traits despite the ploidy-independent genomic structures. DNA methylome analysis in the 32 representative accessions excitingly showed a ploidy-dependent (but population structure-independent) decrease in the genome-wide DNA methylation levels in the CG and CHG contexts, in gene-body and putative gene-promoter regions. This direct correlation of the genome-wide epi-marks to the ploidy-levels suggested the possibility of a dynamic genomic/epigenomic reorganization in polyploid evolution in hardy kiwi, which is reminiscent of so called "genome shock".

## The breeding of the new kiwifruit cultivar 'Ruiyu' with green flesh

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'Ruiyu' is a mid-ripening kiwifruit cultivar, which was developed through hybridization using 'Qinmei' as the female parent and K56 as the male parent. The fruit is long cylindrical with a slightly flattened shape, averaging 90g in weight, with a maximum weight of 128g per fruit. The skin is yellowish-brown with golden-brown hard hairs, and the top of the fruit is slightly convex. On average, each fruit contains around 450 seeds. The flesh is bright green, tender, juicy, and has a deliciously sweet flavor. It has a soluble solid content of up to 21.3%, titratable acidity of 0.82%, total soluble sugar content of 11.55%, sugar-acid ratio of 14.09, and vitamin C content of 78.09mg/100g. The ripening period after harvest at room temperature is 20-25 days, and it can be stored for up to 150 days under refrigeration conditions. The tree has vigorous growth in spring but weak growth in autumn, and it bears early and productive fruiting. In the northern foothills of the Qinling Mountains in Shaanxi, China, the fruits mature in mid to late September. It was approved by the Shaanxi Fruit Tree Appraisal Committee in January 2015 and obtained a certificate of protection as a new plant variety in China in May 2017.

**\*Young mind award**

# Establishing a new cultivar development programme in Italy

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An account of the difficulty, surprise and success in the journey of establishing a kiwifruit new cultivar evaluation programme in a new region. Following a long established and successful programme in New Zealand between Zespri and Plant and Food Research (now under KBC) a decision was made to replicate part of the programme into Italy. The presenter will discuss 4 years of work to achieve this, some initial findings and next steps.

## Breeding on red-flesh and yellow-flesh kiwifruit in Sichuan China

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Sichuan Provincial Academy of Natural Resource Sciences has been committed to the collection, conservation, breeding and utilization of kiwifruit resources for more than 40 years. Since the breeding of the world's first red-flesh kiwifruit 'Hongyang' in the 1990s, new red-flesh and yellow-flesh kiwifruit have been continuously selected through the evaluation of wild kiwifruit resources, selection of seedlings and cross breeding. New cultivars of red-flesh kiwifruit 'Hongshi 2', 'Hongao' and 'Huahong 4' and yellow-fleshed kiwifruit 'Jinshi 1', 'Jinshi 4', 'Huajin 3', 'Jinao' have been bred. In this paper, the breeding background and characteristics of new cultivars were comprehensively introduced. These new cultivars with advantages and potential could lay the foundation for kiwifruit renewal upgrading and industry sustainable development.

## A sweet flavor kiwifruit (*Actinidia eriantha*) cultivar

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Kiwifruit (*Actinidia eriantha*) is a novel berry with great development potential after *A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa*. However, most of the *A. eriantha* fruit are difficult to meet the fresh edible requirement because of their sour flavor and low sweetness, which has been restricting the development of *A. eriantha*. Therefore, sweet flavor is an important breeding trait for *A. eriantha*. Fortunately, we found a sweet flavor genotype (code: 'MM-24') with excellent fruit traits in Nancheng County, Jiangxi Province, P.R. China. Then, we performed DUS test (Distinctness, Uniformity and Stability) during 2017-2019, and the results showed that 'MM-24' is a novel diploid genotype, the three-year average value was 46.5g for its fruit weight, 19.4% (highest was 20.5%) for soluble solid content, 21.75% for dry matter, 9.25% for total sugar content and 0.94% for titrable acid content, 662.6 mg/100 g for ascorbic acid. These traits related sweet flavor of 'MM-24' was significantly higher than the reported *A. eriantha* cultivars, so we named it 'Sweet'. Finally, we assembled the nearly gapless genome of the 'Sweet' cultivar, and using metabolome and transcriptome techniques found that the efficient conversion of starch to sugar is the main reason for the sweet flavor of the 'Sweet' cultivar, and *beta*-amylase gene family members were the main responsible genes.

\*Young mind award

## Kiwifruit trunk disease in New Zealand

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This review gives an account of the current knowledge of kiwifruit trunk disease in New Zealand. First reported in New Zealand in the early 2000s, the syndrome has been known as vine decline, swollen trunk disorder, crown decay disorder, kiwifruit wood decay and leader dieback. Overall, these diseases of the wood of kiwifruit trunks and leaders are currently known as kiwifruit trunk disease (KTD). Symptoms of KTD can include collar rot/crown decay, sparse canopies, small fruit, uniformly swollen trunks, cracking, cankers or discrete bulges of the trunk or leader, and the sudden death of a leader or the entire vine. When the affected part is cut open, the internal wood is discoloured. First recorded in *Actinidia chinensis* var. *deliciosa* 'Hayward' vines over 15 years of age, KTD has now been found on all commercial cultivars. Early research established an association between the fungus *Neobulgaria alba* (syn. *Phialophora alba*) and symptomatic 'Hayward' vines, while species with *Cylindrocarpon*-like anamorphs were found to be commonly associated with rootstock disorder and leader dieback in *Actinidia chinensis* var. *chinensis* 'Hort16A'. More recent research in New Zealand has identified three major groups within the *Nectriaceae* that are associated with KTD; the *Fusarium solani* species complex, the *Ilyonectria* species group, and *Neonectria microconidia*. In addition to these, *Neobulgaria alba* and *Cadophora dextrinospora* (syn. *Mollisia dextrinospora*) are commonly found in the discoloured tissues. A complex of fungi are associated with KTD, and more species continue to be identified within this group. Few of the organisms are known to cause symptoms that can be solely attributed to them, with several groups being associated with each symptom. Directions for future research on the disease and fungal complex should include species distribution, epidemiology and control methods as well as which organisms are the primary pathogens.

# The threat of *Ceratocystis* species to the New Zealand kiwifruit industry

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*Ceratocystis fimbriata* represents a fungal species complex of multiple strains, each with a distinct host range and geographic distribution. Many of these strains, particularly those of the Latin American Clade, cause wilt and canker diseases of economically important plants, including kiwifruit, mango, coffee, cacao and lemon. The first reports of *C. fimbriata* strains causing wilt disease in kiwifruit were reported in Southern Brazil, with some growers reporting vine losses of 20 n 40% as a result of this pathogen. Pathogenicity studies indicate that strains from some other hosts and geographic locations are also highly pathogenic to kiwifruit, and therefore biosecurity efforts must have a broader focus than only those pathways from Southern Brazil. Kiwifruit Vine Health (KVH) is a small organisation dedicated to protecting the biosecurity interests of New Zealand kiwifruit growers, an industry worth \$3B in annual export returns. KVH's pest and disease Risk Matrix ranks *C. fimbriata* as one of the most significant biosecurity threats to the New Zealand kiwifruit industry and therefore readiness work is being prioritised to improve our state of industry preparedness for incursion and response to this pathogen. This readiness work includes activities undertaken in partnership with Biosecurity New Zealand and research funded by Zespri Innovation.

## The high potential of essential oil emulsions in restricting *Pseudomonas syringae* pv. *actinidiae* virulence

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Crop pathogen management is a priority for sustainable agriculture development. Indeed, the large use of pesticides is threatening both human and environmental health, while simultaneously pushing antimicrobial resistance occurrence. Exploiting defence arsenal already present in natural ecosystems represents a promising approach to develop solutions concurrently durable, effective and environmentally neutral. Essential oils (EOs) are naturally occurring compounds displaying a potent antimicrobial activity. Indeed, they can have multiple targets among molecular and structural components of microbial cells. However, their high extraction cost, low stability in the environment and poor water solubility slow down their potential application in crop protection. Modern approaches, like nanotechnologies, can provide solutions to the above-mentioned issues and, at the same time, can outperform the antimicrobial activity of EOs. The nanoformulation of EOs improves their solubility in water, hence their availability, and simultaneously can prevent their degradation in a natural environment like open fields. In this context, this work aimed to develop and test nanoformulation of single and/or multiple EOs combining different antimicrobial activities to provide a durable control efficacy against *Pseudomonas syringae* pv. *actinidiae*, the causal agent of kiwifruit bacterial canker. We investigated i) the activity of 4 different EOs, single or combined, against *P. syringae* pv. *actinidiae*, in-vitro for pathogen growth inhibition and biofilm formation prevention, ii) their encapsulation using different formulations, and iii) their efficiency in reducing disease index and severity on kiwifruit plants in pilot trials in field conditions.



## Investigating a new Psa variant in New Zealand

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A new genetic variant of the kiwifruit vine pathogen *Pseudomonas syringae* pv. *actinidiae* (Psa), the cause of bacterial canker in kiwifruit (*Actinidia* spp.), was identified in New Zealand during 2022. The variant, which is missing the hopF2 effector gene, was detected as part of routine testing of Psa isolates collected from two commercial orchards in New Zealand. A specific PCR primer was developed and the Psa variant was tested in a competition assay on tissue cultured *A. chinensis* var. *chinensis* 'Hort16A' and *A. chinensis* var. *deliciosa* ('Hayward') plantlets and found to out-compete wild-type Psa strains on 'Hort16A' but not on 'Hayward'. The competition assay was repeated using *A. chinensis* var. *chinensis* 'Zesy002' (commonly known as Gold3) and 'Zes008' (commonly known as Red19) tissue culture plantlets. The hopF2 Psa variant did not out-compete wild type Psa on 'Zesy002' but did on 'Zes008', although to a much lesser extent than on 'Hort16A'. The two orchards of interest, where the original hopF2 Psa variants were first detected, and nearby properties, had additional leaf samples collected to monitor the persistence and spread of the variant. Trap plants were also utilised to increase the recovery of Psa from the two orchards of interest by suspending potted 'Hayward' plants beneath the vine canopy during predicted rainfall events in spring 2022. Leaves that developed typical Psa spotting were collected, and Psa was isolated and tested using the new primers. Freshly collected strains of Psa (259) were screened and the variant was detected in just 6.6% of these. Research findings to date suggest the newly emerged hopF2 Psa variant is not significantly more pathogenic on 'Zesy002' and 'Zes008' following inoculation studies on leaves and stems of potted plants in containment glasshouses. On going monitoring and vigilance is recommended.

# Economic Impact of Fruit Fly incursions in the Bay of Plenty

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Fruit flies are among some of the most destructive pests in the world and are considered one of the kiwifruit industry's "Most Unwanted" biosecurity threats. New Zealand is one of the few countries in the world free from economically significant fruit flies, such as Queensland Fruit Fly, Mediterranean Fruit Fly and Oriental Fruit Fly. This pest freedom status provides a significant advantage to New Zealand growers as it enables continued access to our export markets. Since 1989, fruit fly has been detected within New Zealand borders on 12 occasions, of which three resulted in significant eradication responses to successfully prevent establishment of the pest: Mediterranean fly in 1996 (Mount Roskill, Auckland) and two Queensland fruit fly incursions in 2015 (Grey Lynn, Auckland) and 2019 (Devonport and Northcote, Auckland). A new study commissioned by Zespri Innovation modelled three different Fruit Fly incursion scenarios (a base, medium and large scenario) and estimated the effect of an incursion in Te Puke (BOP) would have on the New Zealand kiwifruit industry. The model used data from the previous Auckland fruit fly responses to model impacts across a single season for SunGold and Hayward kiwifruits, assuming a successful eradication. The cost of the three incursion scenarios ranged from nearly \$200 million losses in the base scenario to almost \$695 million in the large scenario. This model highlights the importance of detecting an incursion early as the sooner an exotic fruit fly is reported, the quicker a response can be initiated, ultimately limiting the impacts to the New Zealand kiwifruit industry.

## Optimising biocontrol for brown marmorated stink bug (BMSB) in kiwifruit

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The brown marmorated stink bug (BMSB, *Halyomorpha halys*, Hemiptera: Pentatomidae) is one of the most unwanted agricultural insect pests worldwide. They are native to East Asia but repeatedly intercepted at the New Zealand border and listed as a major threat to the country's agricultural and horticultural industries, which include kiwifruit. *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is considered as the most promising natural enemy for biocontrol of BMSB. Adventive populations of *T. japonicus* occur in the United States, Italy, and Switzerland, where classical biocontrol releases are already underway. In Aug 2018, NZ EPA approved the conditional release of *T. japonicus* should a BMSB incursion occur in NZ. However, information on the biology and release strategies of *T. japonicus* in order to maximise its effectiveness against BMSB is still lacking. This work aimed to conduct several field studies in its native range in Mei County, Shaanxi province, China, to optimise release numbers of *T. japonicus*, timing and frequency in kiwifruit orchards. Field studies were conducted in experimental kiwifruit orchards in field cages. Information from such studies was further used to plan and conduct experimental releases to assess impact on BMSB populations and feeding damage in kiwifruit. Results from release ratio trials showed that releasing one wasp against one BMSB egg mass was more effective (92% parasitism observed) than one wasp against two (63.3% parasitism) or three (30.1%). Release frequency experimental results using mesh cages showed that three consecutive releases (one release per week) achieved the highest parasitism results (89.5%). Results from field experimental releases of *T. japonicus*, where parasitoids were released in May 2022 during three consecutive weeks (one release per week) showed that mean parasitism over 3 weeks was significantly higher (38.9%) in release plots than non-release plots (12.3%). The average incidence of damaged kiwifruit was 21.6% from June to September in release plots, which was significantly lower than that on non-releasing plots (40.4%). These results show great promise for developing a biocontrol strategy against BMSB in kiwifruit for long-term management.

## Trial of hydroponic cultivation of kiwifruit using wet tolerant rootstock, *Actinidia macrosperma*

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Hydroponics is beneficial for increasing the productivity of certain horticultural crops. Normally, *Actinidia* spp. are not suitable for hydroponics due to their low wet tolerance. In this study, highly wet tolerant species, *A. macrosperma*, was used as a root stock to attempt deep hydroponic cultivation of kiwifruits. Two-year-old 'Kagawa UP-Ki5' (interspecific hybrid cultivar between *A. rufa* and *A. chinensis*) grafted on *A. deliciosa* 'Hayward' and *A. macrosperma* [Fuchu] were grown in the deep-water hydroponic conditions. Vines grafted on *A. macrosperma* continued to grow, while those on *A. deliciosa* rootstock stopped growing and died within 3 months. In addition, hydroponic cultivation was also applicable to 2-year-old *A. deliciosa* 'Hayward' and *A. chinensis* [Rainbow Red<sup>®</sup>] scion vines grafted on *A. macrosperma* rootstocks. After the first fertilization, salt secretion from leaves and leaf browning occurred in the young vine of 'Kagawa UP-Ki5' grafted on *A. macrosperma*, although the vines resumed growing soon. The timing of the first fertilization at 0, 4, and 6 weeks from the start of hydroponic cultivation had no effect on the occurrence of these symptoms. After the 2 years of hydroponic cultivation, more than 300 fruits were harvested from 4-year-old 'Kagawa UP-Ki5' grafted on *A. macrosperma*. The fruits characteristics were equivalent to those harvested from the vine grown in soil. These results indicate the feasibility of deep hydroponic cultivation and fruit production of kiwifruit using *A. macrosperma* as a rootstock. Further optimization of cultivation conditions is expected to improve productivity.

**\*Young mind award**

# Covered kiwifruit cultivation on the axis of climate change

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Climate change causes many negative effects on horticultural crops and especially on species that need more water, such as kiwifruit, as in all branches of agriculture. Growers are in a difficult situation especially in the face of increasing temperatures, scarce water resources and sudden climatic events. One of the most important protection methods applied today to protect from these negative effects is to cover the orchards/vineyards with different plastic materials. Different plastic covers developed for this purpose have the potential to contribute to the increase in quality as well as protect the kiwifruit plant from the negative effects of the climate. Using the covering systems is gradually increasing in response to the increasing high costs in kiwifruit growers in different countries around the world. Although these practices increase the establishment costs of the orchards, it is also due to the fact that they oblige the growers to use these covering systems in increasingly risky conditions. However, it is possible to reduce these high costs in favour of growers by increasing fruit yield and fruit quality. For this purpose, cultivating the new yellow and red fleshed kiwifruit cultivars using covered systems not only can help to protect from the negative effects of the climate, but also can help to increase their quality. Covered or closed kiwifruit production systems, which are common in the Far East, are increasing in Europe, especially in Italy. Even in the south of Italy, which is very hot in summer, kiwifruit production has become possible with the help of covered system production. Research on the effects of these systems on fruit quality is ongoing and more precise information on this subject will be obtained in the near future. These results show us that it will be possible to grow kiwifruit in marginal areas thanks to the use of covered systems, despite the negative effects of climate change in the coming years.

## Effects of kiwifruit rootstocks on physiological responses of grafting combinations under waterlogging stress

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Kiwifruit vines are known to be sensitive to waterlogging stress, which is increasingly occurring at orchard level as an effect of global climate change, which alters precipitation patterns in many areas, and improper irrigation management. Anaerobic conditions induced by waterlogging create an unfavorable environment for the roots, contributing to an early decline in roots activity and functionality. The search for rootstocks that increase the waterlogging stress tolerance of kiwifruit vines appears to be an efficient complementary strategy to pursue, but little is known about the behavior of different rootstocks under waterlogging stress. In the present study three grafting combinations grown in pots were compared to investigate the responses of the kiwifruit scion cultivar Zesy002 (*Actinidia chinensis* var. *chinensis*) when grafted on Hayward, D1 (*A. chinensis* var. *deliciosa*), and Bounty 71 (*A. macrosperma*) during waterlogging stress. The physiological parameters were measured and visual symptoms on the leaf and root system were monitored during the experimental trial that consisted of a 9-day waterlogging stress phase. Preliminary results showed that waterlogging conditions induced a decline in the physiological parameters such as net photosynthetic rate, stomatal conductance and transpiration, a progressive reduction in the leaf area due to leaf crumpling and upward curling, browning of roots and an inadequate formation of feeder absorbing roots in the layers most affected by water stagnation. Among the three grafting combinations, Zesy002 /Bounty 71 was identified as the least affected by waterlogging stress, maintaining higher values of photosynthesis and stomatal conductance for a longer time and showing no visible effects of the waterlogging stress on the root system compared to the other grafting combinations. These preliminary results increase knowledge about the influence of rootstocks on the physiological processes of the grafted cultivar and the rootstock-scion interactions, demonstrating that the Bounty 71 rootstock can be effective in improving waterlogging tolerance of grafted kiwifruit vines by regulating physiological responses and limiting the negative effects imposed by waterlogging.

## Precision irrigation strategy based on a feedback adjustment mechanism by soil moisture monitoring in kiwifruit orchard

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Mediterranean area is characterized by an high evapotranspiration environment, with high light intensities and air temperature, low precipitation during summer and relatively high vapor pressure deficits. Under these growing conditions, kiwifruit irrigation management should be as accurate as possible in order to avoid over-watering, which usually occurred under empirical management (conventionally applying approx. 7000-10000 m<sup>3</sup>/ha), and also water deficit. Under the scenario of climate change affecting water cycle in several geographical areas, the developing of a proper irrigation management in kiwifruit is needed. The study aims to develop a precision Irrigation Strategy based on a water-balance method with a feedback adjustment mechanism using continuous soil moisture measurements. The irrigation strategy allowed to adapt irrigation volume to actual vine water requirement and ensure optimal soil conditions for root development, avoiding the establishment of conditions predisposing degenerative phenomena of the root system. A two year-field experiment was conducted in a commercial and mature kiwifruit orchard (*Actinidia chinensis* var. *chinensis*) located in Metaponto (South Italy) and irrigated with sprinkler system. Precision irrigation strategy was compared with the conventional management. Soil moisture probes was installed at different depths (10-60 cm) to continuously monitor the water content along the soil profile. Irrigation volumes were adjusted daily in order to keep the soil moisture between the field capacity (FC) and readily available water (RAW), defined as 30% of the total available water (AW) for kiwifruit. The daily irrigation volume was applied in three/four irrigation interventions in order to assure an optimal water condition and management of the upper soil layers (0-30 cm), reducing the midday depression. The seasonal theoretical irrigation volume calculated through a simplified water balance using FAO Kc, was 8,619.6 m<sup>3</sup>/ha. Data obtained from probes were evaluated and used to adjust weekly the theoretical volume allowing reductions of the irrigation volume of 36% for the precision irrigation strategy. A more efficient and sustainable irrigation management could result from water-balance-based irrigation scheduling with a feedback adjustment mechanism using continuous soil moisture monitoring and the partition of the daily irrigation volume into more interventions.

## Kiwifruit production in a tropical highland of Mexico-soil and climate

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There is little information regarding kiwifruit production in the tropics. Green and yellow-fleshed kiwifruit (*Actinidia chinensis*) are deciduous climbing shrubs that grow well in temperate countries and blooming requires between 300-900 chilling hours (CH) below 4°C in winter. In the tropical highlands during winter there are days with temperatures of about 28°C and selected kiwifruit vines originated by seeds attain 50% of natural bud burst, producing good quality fruit. The kiwifruit orchards are in Huatusco, Veracruz, Mexico, at 1848 m of altitude, and close to the kiwifruit orchards at 900-1300 m of altitude coffee beans (*Coffea arabica*) are produced. The objectives of this study were to show that kiwifruit would be produced in the tropics and that is an alternative to small producers with low income. The kiwifruit vines grow on soils of volcanic origin with high levels of carbon (6.95%) and organic matter (12%), and optimum available nitrogen content (34 ppm). The bulk density was 0.81 Ton m<sup>-3</sup>, and pH 5.3. The kiwifruit orchards are not irrigated and the annual rainfall is between 1100 and 1600 mm. The average temperature was between 12-14°C in the coldest months (November-February) and 15-18°C in the warmer months (March-September). According to Da Mota's CH quantification model, this zone accumulates 443 CH. Most of the kiwifruit consumed in Mexico is imported and it is expensive for people with low economic input. The production of selected kiwifruit produced in tropical highland conditions of Mesoamerica could initiate new business for small farmers and currently is a new strategy for farmers to diversify their diet and economy. Kiwifruit growth information out of traditional areas of production is valuable due to the consequences of climate change.



## Water and nutrient balances on New Zealand's kiwifruit orchards

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The kiwifruit industry has the opportunity to contribute to the advancement of global sustainability goals including freshwater protection. Furthermore, many governments, including New Zealand's, are developing new science-based regulations in primary industries to strengthen environmental protection while maintaining socio-economic development. Currently there is almost no robust data to confirm the water and nutrient needs of New Zealand kiwifruit orchards, especially for yellow fleshed kiwifruit. Zespri is working with Plant & Food Research (PFR) to fill this knowledge gap. This paper presents preliminary analysis of results from a field study to quantify the water balances and nutrient use efficiency in kiwifruit orchards. Equipment has been installed on nine orchards that span a range of different soil types (allophanic and pumice), different cultivars (*Actinidia chinensis* var. *deliciosa* 'Hayward' and *A. chinensis* var. *chinensis* 'Zesy002' (commonly known as Gold3), and different management practices (organic and conventional). All monitoring is being done on commercial kiwifruit orchards. The soil water content is measured using TDR (time domain reflectometry), vine transpiration is measured with sap flow sensors, drainage is measured with passive-wick samplers, and overland flow is recorded using drainage channels with automatic proportional samplers. Data will be presented to illustrate the complex nature of drainage and runoff events, and to show the impacts of reducing nitrogen inputs. Information from these field experiments is being used by Zespri to help the industry to better manage irrigation and fertilizer usage in a more efficient and therefore sustainable way.

## Degreening of the yellow-flesh kiwifruit (*Actinidia chinensis* cv. Dori) reduces chilling injury after storage at 0°C

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World production of kiwifruit is expanding rapidly with the introduction of new yellow-flesh cultivars of *Actinidia chinensis*. Some of these new cultivars are susceptible to chilling injury, reducing their storage life and quality at point of sale. The yellow-flesh kiwifruit cv. Dori was recently demonstrated to be susceptible to chilling injury during commercial storage at 0°C. Here we evaluate the possibility of using an earlier harvest, in conjunction with a degreening treatment at 15°C prior to storage at 0°C as alternative to reduce chilling injury. Fruit of cv. Dori was harvested one week before the commercial harvest (at 104° hue), then degreened for 0, 7, 14 or 21 days at 15°C, and stored for 60 days at 0°C. To simulate commercial practice, other fruit was harvested one week later (at 100° hue) and stored at 0°C. Another batch of fruit was stored at 5°C to determine a possible 'safe' storage temperature for Dori that avoided chilling injury. Fruit quality was evaluated during ripening at 20°C for twelve days every three days. Chilling injury appears during ripening with higher incidences in the early-harvested fruit. Early harvest, in conjunction with degreening at 15°C, reduced the susceptibility to chilling injury, being absent with 14 or 21 days similar to that in fruit stored at 5°C. The degreening and fruit softening rates were positively related to the length of time at 15°C before storage at 0°C. The highest rate of softening (to < 10 N after 6 days at 20°C) was in fruit stored throughout at 0°C and was independent of harvest time. Postharvest storage at 5°C avoids this problem but results in premature softening, eliminating this as a commercial option. Early harvest, together with degreening at 15°C, significantly improved flesh color, while reduced chilling injury allowed fuller expression of optimal sugar: acid balance. Further investigations should fine-tune these relationships and so result in the development of an optimal commercial protocol for harvest timing and postharvest temperature management in Dori.

## 'SunGold' kiwifruit ethylene production in response to controlled scuffing

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Kiwifruit (*Actinidia chinensis* var. *chinensis*), once harvested, are exposed to potentially injurious situations throughout the packing line and transportation. These injurious situations involve forces which can induce mechanical damage. Mechanical damage can induce irreversible injury to the skin as well as the internal tissue of the fruit. Specifically, mechanical damage can stimulate physical or physiological changes in fruit. Particularly, damage can stimulate ethylene biosynthesis and this increase in biosynthesis can result in ethylene contamination in storage and within packaging. Ethylene contamination is associated with premature softening, with concentrations as low as 30 ppb eliciting ripening responses in kiwifruit. Thus, this work investigated whether the mechanical force of friction could stimulate ethylene biosynthesis. Friction-induced skin injuries like that observed in bulk bin transport were induced through controlled scuffing. Scuffing results in the injury known as superficial skin rub (SSR), which can vary in severity. To capture a range of severities 'SunGold' kiwifruit were harvested from one orchard on five occasions (ISO weeks 12, 14, 16, 18, and 20) and half the fruit received controlled scuffing. Ethylene production in scuffed and non-scuffed fruit was measured over a period of 24 h with an ETD-300 at 20°C immediately after scuffing. Ethylene production was also measured twice over a three-week storage period (1°C) for fruit from ISO week 18. Ethylene production of the treatments fluctuated with harvest time, with significant differences observed between scuffed and non-scuffed fruit. Scuffed kiwifruit with SSR symptoms exhibited increased ethylene production when compared to non-symptomatic fruit. However, among symptomatic fruit, the severity of SSR injury had minimal impact on the ethylene production. Controlled scuffing has shown that skin injuries caused by friction can stimulate ethylene biosynthesis. Particularly, this work suggests that kiwifruit injured to the point of symptom expression may cause issues in storage and marketing if not removed. However, repeat experiments of this phenomenon with more fruit and severities would be required to build confidence in this result.

## Quality aspects of the kiwifruit core

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The core of a kiwifruit is a significant, but often overlooked, component of the fruit. It may comprise 2–10% of the fruit, depending on genotype. The core is composed of isodiametric cells which contain significant amounts of starch, with the ventral vascular bundles along the length of the outer tissues of the core. The core is usually white, forming a significant element of the appearance of the sliced fruit. It is an obvious component when the fruit is cut open, both in its appearance and texture. Both these characteristics may be problematic to the quality of the fruit. In some genotypes the core may darken when ripe. Also, when ripe, an unusually firm core relative to the rest of the fruit results in what is termed a 'hard core', identified as a disorder potentially reducing the eating quality. This situation has variously been ascribed as an effect of controlled atmosphere storage or treatment with 1-methylcyclopropene, yet there are numerous examples that show this not to be the case. Observation of a range of genotypes has shown the basis for hard cores may start with a temporal separation between the ripening (softening) of the outer pericarp and core, with the start of core ripening occurring in some instances weeks after the outer pericarp. This genetic basis is the baseline from which postharvest treatments may further influence the occurrence of ripe fruit with hard cores.

## Molecular basis of tissue-specific kiwifruit acclimation to post-harvest cold stress

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Kiwifruit is a climacteric fruit with high ethylene sensitivity and respiration rate, which leads to shorten postharvest life due to rapid softening and senescence. Postharvest handling of kiwifruit focuses extensively on low-temperature storage for preservation of fruit quality and elongation of its lifespan. However, cold storage can cause postharvest losses attributable to prolonged cold stress on kiwifruit, which is susceptible to cold exposure, due to its subtropical origin. The aim of this study was to understand the acclimation mechanism of kiwifruit (*Actinidia chinensis* var. *deliciosa* cv. 'Hayward') to cold stress during storage using multi-omics approach. For this purpose, kiwifruit was harvested and subsequently cold stored (0°C and 95% RH) for. To achieve different levels of cold stress responses, the kiwifruit was exposed to low temperature (0°C) for different periods, namely 1 (H), 15 (S1), 30 (S2), 45 (S3), 60 (S4), 75 (S5) and 90 (S6) days. The assessment of fruit ripening status was performed in both pericarp and columella tissues at harvest (H) and during cold exposure (S1-S6). Anatomical disturbances, biochemical indicators and physiological data revealed that fruit at S1 and S6 stages showed substantial changes in cold-derived cellular damage. Wide metabolomic, proteomic, transcriptomic and whole-genome bisulphite sequencing along with integrated bioinformatic analysis were performed in cold stress pericarp and columella tissues at various timepoints. This system-based approach revealed cold-associated transcriptional, epigenomic, proteomic and metabolic signatures and identified pathways modulated by the common or distinct hallmarks in pericarp and columella tissues. In addition to this, computational models allowed us to identify major transcription factors (TFs) that involved in the acclimation of kiwifruit to cold environmental. Using several genetic approaches, the biological relevance of this TFs was evaluated. Overall, this work provides a refined blueprint of the molecular acclimation of the kiwifruit tissue to cold storage, thereby enabling our ability to control postharvest fruit handling. This work was supported by European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH– CREATE – INNOVATE (project code: T2EAK-03007; Premium Kiwi).

# Conceptual and mechanistic framework for prediction of kiwifruit quality in storage over time

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The postharvest sector seeks to minimise fruit deterioration between harvest and the consumer. Extensive research has been conducted to identify and manage factors responsible for any reduction in quality occurring during storage. However, studies have primarily examined individual factors and specific defect types in controlled environments, leaving the overall dynamics and interactions unclear. Understanding the interplay between different defects is crucial, as they may directly or indirectly amplify the incidence or severity of one another over time. Continuous collection of empirical data for the entire factor-defect system is impractical due to the interventions required by most quality control technologies being disruptive to the postharvest system's natural flow. In considering this challenge, a conceptual modelling approach is proposed that integrates the range of physical, physiological and pathological factors to develop a mechanistic/probabilistic framework for the dynamic factor-defect system. Within this framework, poor vine condition, incorrect harvest maturity and poor handling are identified as three major at-harvest aspects associated with subsequent fruit defects. The set of defects considered includes green fruit, storage stains, rots, and softening with soft patches. The incidence and severity of each damage element not only vary over time (from early to mid to end of the season) but are also influenced by the interactions and internal dynamics of the other damage elements within the tray, batch, or pallet, both deterministically and probabilistically. This is translated to a Directed Acyclic Graph (DAG) that can be utilised in experimental designs where specific interactions or dynamics within the controlled environment need to be studied. By adopting this conceptual approach, we aim to enhance our understanding of the complex factor-defect system, enabling more informed decision-making and improved practices and risk management within the postharvest sector.

# Understanding the influence of postharvest storage conditions on kiwifruit using RNA-Seq transcriptome profiling

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Disorders that develop during long-term low-temperature storage of kiwifruit can result in significant postharvest losses. However, it is debated how similar the underlying biochemical and physiological processes associated with different disorders, such as chilling injury, freezing damage and postharvest ripening during storage are to each other. RNA-Seq transcriptome profiling is a sequencing technique that can be used to show the presence and quantity of RNA in biological samples. It has the power to elucidate some of the complex gene expression changes and therefore the underlying molecular/biochemical processes that occur in kiwifruit during storage. In this talk we will highlight some of the key differences found in kiwifruit under chilling, freezing and ripening storage conditions. A better understanding of this will allow for the development of ways to mitigate the amount of fruit lost during storage and to better understand what processes are occurring in kiwifruit postharvest.

**\*Young mind award**

# DNA methylation reprogramming during the transition from winter dormancy to growth resumption in kiwifruit

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DNA methylation is an important epigenetic modification that plays a significant role in developmental transitions by regulating gene expression. In kiwifruit, *Actinidia chinensis*, the transition from winter dormancy to growth in spring is a critical phase, setting the stage for flowering and fruit development in upcoming seasons. It is largely unknown if this process is associated with changes in DNA methylation. In this study, we found the application of a hypo-methylating agent, 5-azacytidine on dormant buds significantly accelerated budbreak in kiwifruit. We generated a single-base resolution map of CG, CGH and CHH methylation by whole-genome bisulfite sequencing (BS-seq) and performed a transcriptome analysis to compare the buds in a dormant state in winter and at growth resumption in spring. We found genome-wide CHH hyper-methylation in dormant buds and much reduced CHH methylation at the stages when vegetative growth and reproductive development are reestablished. Many genes involved in growth and flowering, including the genes regulating methylation and demethylation, were coordinated with the amount of DNA methylation. Surprisingly, CHH hypomethylation during the transition from winter dormancy to growth resumption was accompanied by an down-regulation of transposon (TE) expression. Elevated expression of the RdDM pathway genes detected in active growing buds may suppress this harmful TE activity, while allowing genome-wide chromatin and gene expression reprogramming during transition from dormancy to the actively growing stage.



## Unravelling the genetic control of budbreak in kiwifruit (*Actinidia chinensis*) – analysis of a cold-responsive FLC-like gene

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Many temperate perennial plants need a prolonged exposure to cold temperatures during winter to recommence growth in the subsequent spring. The cycles of growth and dormancy are controlled by intricate genetic regulatory networks and may be influenced by epigenetic mechanisms, although the specific genes and mechanisms involved are not yet well understood. In this study, we conducted RNA-Seq analysis on the buds of the woody perennial vine kiwifruit (*Actinidia chinensis*) under both natural field conditions and controlled environments to investigate how seasonal changes and chilling impact dormancy and growth regulation. During this investigation, we identified and characterized a MADS-box gene that shares similarity with Arabidopsis FLOWERING LOCUS C (FLC). Notably, we observed elevated expression of the AcFLC-like (AcFLCL) gene during bud dormancy and in response to chilling. Additionally, we discovered long non-coding (lnc) antisense transcripts with expression patterns that were contrary to AcFLCL, along with shorter sense non-coding RNAs. Furthermore, exposure to cold temperatures led to an increase in trimethylation of lysine-4 of histone H3 (H3K4me3) at the 5' end of the gene, indicating the presence of multiple layers of epigenetic regulation in response to chilling. Using transgenics, we found that overexpressing AcFLCL in kiwifruit accelerated budbreak, while gene editing using CRISPR-Cas9 resulted in transgenic lines with significantly delayed budbreak, suggesting that this gene plays a crucial role as a growth activator. These significant findings have important implications for the future management and breeding of perennial plants, enabling us to enhance their resilience to a changing climate.

## Enhancing budbreak in kiwifruit: study of biological mechanisms involved in dormancy release and alternative potential products to the Hi Cane

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In perennial plants, dormancy stops vegetative growth permitting to save energy and to be protected when environmental conditions become unsuitable or dangerous. Several exogenous and endogenous factors are involved in dormancy initiation and its end. Winter chilling period and its characteristics (temperatures, duration etc.) is a key environmental component for satisfying chilling requirement, which is known to be the main natural force activating budbreak processes and vegetative/reproductive resumption in spring. During plants' dormancy onset and release, several endogenous pathways have been hypothesized to play a role: gene transcription, hormonal balance, primary metabolism, oxidative stress, and signalling, mineral nutrient balance. In addition, increasing evidence showed that exposure to stressful conditions could activate epigenetic modifications. This plant epigenetic "memory" is relevant in perennial species enabling phenotypic plasticity and adaptation to unfavorable environments becoming also transmissible to "progeny" (cloned or sexually reproduced). When environmental conditions are instable, could not assure the achievement of chilling requirement and/or regular and strong budbreak, Hydrogen Cyanamide (HC) is applied. It is a powerful dormancy breaker, commonly used during the past years in kiwifruit to increase budbreak (BB) and flowering, to provide compact bloom and to decrease unwanted lateral flowers. Its application has been banned in many countries because of its danger to humans. Unfortunately, it is not fully understood which endogenous mechanisms are involved in the HC induced budbreak in kiwifruit. Research made on alternative dormancy breakers show that they are typically less effective than HC and less stable across time, regions and cultivars. A complex study of the dormancy related processes has been started in Italy. On the one hand, a three-years field experiment with alternative to HC budbreakers has been carried out in two different locations: Metapontino area (Basilicata, Southern Italy) and Latina (Lazio, Central Italy). A restricted number of tested products have shown interesting characteristics as potential substitutes for HC treatments for both, Hayward and Zesy002 cultivars. In general, those products have been evaluated as more sensible to application period (Early, Mid and Late applications), but in case of optimal treatment time, the results were comparable to HC in terms of budbreak homogeneity (timing and number of king flowers per winter bud), bloom compactness (around one week) and lateral flower thinning (reducing cost of manual operations). At the same time, a tool to choose the best time of treatment has been evaluated (Hazel Trex® test). On the other hand, an in-depth molecular study of endogenous pathways involved in kiwifruit budbreak (transcriptomic, metabolomic, hormonal and mineral balance) has been started in addition to the field trial. Therefore, the study aims to characterize the dormancy outcome (with and without HC application), evidencing main endogenous factors involved. A deeper understanding of these interplaying and interconnected mechanisms could open new crop management and breeding solutions in kiwifruit. Further work should be done to achieve study's goals: to improve our understanding of budbreak process in kiwifruit in general and specifically to hypnotize how kiwifruit orchards should be managed in an efficient way in absence of HC.

## Comparative estimation of chilling and heat requirement in five kiwifruit varieties and exploitation of this knowledge for the effective application of a breaking dormancy agent ( Brecaut®) in cv Jintao®

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Kiwifruit cultivation in Italy has spread in warmer areas in the last decade since the low risk of bacterial cancer infection. The rapid introduction of a new specie and cultivars in this area caused, in some cases, problems related to ununiform bud breaking, late frost injury and inconstant productivity. For this reason, it is of great interest to study the thermic requirements of the new cultivars introduced and possible strategies facilitating bud break and blooming. Chilling requirement needed to overcome endo dormancy in deciduous trees is variety specific. It is crucial, particularly for warm areas, to know this feature in order to match properly the plant requirement with the chill accumulation of the area. Once chilling requirement has been satisfied heat accumulation allows trees to overcome eco dormancy which is crucial to avoid the risk of late frost damages. Several approaches have been proposed for estimating thermic requirement and accumulation and their accuracy changed according to their complexity and to the area where they were applied. In this research three methods to estimate chilling accumulation were compared: Chilling hours (CH), Utha Model (CU) and Dynamic Model (CP); and chilling requirements were estimated on 5 kiwifruit varieties belonging to *A. chinensis* and *A. deliciosa* by means of the forcing protocol. After 2 years of observations, the most accurate model resulted CP. *A. chinensis* cultivar reported lower CR than *A. deliciosa* and opposite results were found for heat requirement. The breaking agents Brecaut® was tested on Jintao cultivar in Southern Italy investigating two factors: application time, in terms of percentage of chilling requirement satisfied, and dosage. The application within a windows of 70 and 85% of satisfied chilling requirement at standard dosage appeared appropriate. The higher dosage (150%) increased the bud fertility in the basal part of the shoot.

## Study of nutritional quality and functional genes in kiwifruit

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The kiwifruit is a perennial deciduous vine that belongs to the genus *Actinidia*. The genus *Actinidia* has 75 taxa, comprising 54 species and 21 variations, as of the most recent classification. According to the fruit flesh color of kiwifruits, they are mainly divided into three categories: green-fleshed, yellow-fleshed and red-fleshed. More than 90% of cultivated kiwifruits are green and yellow-fleshed varieties, while red-fleshed kiwifruits have become increasingly popular with consumers in recent years. We estimated chlorophyll, carotenoid, flavonoid, carbohydrates, protein, lipid, dietary fiber, soluble sugar, organic acid, vitamin and aroma compound contents according to different flesh colors and species in kiwifruits. The sugar/acid ratio of red-fleshed kiwifruits was significantly higher than green-fleshed and yellow-fleshed kiwifruits. Moreover, red-fleshed kiwifruits had the highest contents of anthocyanins, total flavonoid, carbohydrate and vitamin B1. In order to further study the anthocyanin accumulation in red-fleshed kiwifruit, we identified a transcription factor AcMYB88, with the similar expression pattern to *AcMYB110* in different developmental stage of red-fleshed 'Donghong' kiwifruit. The results showed that *AcMYB88* was a positive regulator of anthocyanins accumulation. Meanwhile, it could synergistically regulate with *AcMYB110* and *AcbHLH42* to promote the accumulation of anthocyanins. This study provides a scientific evidence for kiwifruit consumption and utilization, and facilitates further revealing the function of MYB transcription factors in the regulation of anthocyanin metabolism in kiwifruit.

# Amyloplast biogenesis and differentiation in kiwifruit

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The biogenesis and differentiation (B&D) of amyloplasts contributes to fruit flavor and color. Here, remodeling of starch granules, thylakoids and plastoglobules was observed as kiwifruit ripened. A novel protocol was developed to purify starch-containing plastids with a high degree of intactness and the amyloplast B&D was studied using label-free (LF)-based quantitative proteomic analyses. Over 3,000 amyloplast-localized proteins were identified, > 98% of them were quantified, defined as kfALP (kiwifruit Amyloplast Proteome). The kfALP data were validated by Tandem-Mass-Tag (TMT) labelled proteomics. Based on the proteome data, amyloplast B&D appeared to be associated with 1) a conserved marked increase in abundance of proteins participating in starch synthesis/degradation during amyloplast biogenesis/differentiation in both of the yellow-fleshed 'Hort16A' and green-fleshed 'Hayward'; 2) up-regulation of proteins for chlorophyll degradation and of plastoglobule-localized proteins associated with chloroplast breakdown and plastoglobule formation during amyloplast differentiation; 3) constant expression in ATP supply and protein import, but continuative reduce capacity in ribosome assembly during amyloplast B&D. Interestingly, two different pathways of amyloplast B&D were observed in different cultivars. In 'Hayward', significant increases in abundance of photosynthetic- and tetrapyrrole metabolism-related proteins were observed, but the opposite trend was observed in 'Hort16A'. In conclusion, this comprehensive proteomic study provides new insights into the potential mechanisms underlying amyloplast biogenesis and differentiation in contrasting kiwifruit with relevance to key fruit quality traits.

# Flavoromics: a new approach to understanding kiwifruit flavour, colour and texture

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As consumer preferences continue to evolve, there is an increasing demand for improved fruit varieties that meet these changing demands. Flavour, colour and texture are the main drivers that influence consumer perception and preferences of foods. Flavour has been defined as a complex multimodal sensation in which different stimuli are observed and interpreted together to generate a unique perception. Understanding flavour perception is not an easy task for researchers since it is derived from hundreds, if not thousands, of chemical compounds responsible for aroma (ortho and retronasal), taste, aftertaste and mouthfeel sensations. Additionally, the texture of food products is also an important attribute that influences consumer choice significantly, while colour contributes to the consumer appeal and acceptance. Flavoromics, a novel interdisciplinary approach, has emerged as a powerful tool to unravel the intricate mechanisms that underlie the complex sensory attributes of foods and beverages. Flavoromics is an untargeted data driven approach to study the chemical basis of flavour, utilising analytical chemistry, sensory and data sciences to generate new insights. Previous work has generated a myriad of information connecting flavour to composition and genetics, however progress has been slow and incremental. Flavoromics presents a key opportunity where our science can lead to better understanding by connecting chemistry, physics and psychological aspects responsible for consumer perception. Here, we provide examples from published studies how flavoromics has been applied to different horticultural crops while discussing the opportunities this approach can have to advance breeding strategies and basic understanding of kiwifruit flavour development.

## Uncovering the effect of knocking down sugar transporters in kiwiberry

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Sugars are essential carbohydrates that affect fruit sweetness and flavour, a key breeding trait that is sought after by most consumers. The economic value of fruits is therefore linked to accumulation and storage of sugars. However, the key players in sugar transport and the molecular mechanism by which sugar is loaded onto the phloem from the leaf and unloaded to the fruit remain elusive, particularly in kiwifruit. Sugar transporters are vaguely classified into three main groups: the monosaccharide transporter-like (MST) gene family; the sucrose transporters (SUCs); and the Sugars Will Eventually be Exported Transporter (SWEET). Many have been implicated in sugar transport, but none have been functionally characterised in *Actinidia* species. We have examined the role of sugar transporters in phloem loading and unloading in *A. arguta* fruits using RNAi lines. We will present how perturbation of these transporters may contribute to changes in accumulation of sugars and some metabolites, and how other aspects of fruit physiology and development are affected. We believe that these findings may provide opportunities for the development of new strategies introduced into breeding programmes aimed to enhance fruit sweetness and flavour.

## Explainable AI-based cis-decoding spots kiwifruit-specific fruit ripening regulatory networks

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Kiwifruit ripening is triggered by ethylene production/treatment, as well as in other climacteric fruit crops. Thus, attempts have been made to identify potential key genes for kiwifruit ripening, based on the known regulatory pathways or genes in "model" fruit crops, including tomato or Cucurbit species. However, recent high-quality genome sequencing and evolutionary aspects have gradually revealed the possibility that the expression networks might have evolved in a lineage-specific manner, depending on variations of cis -regulatory elements (CREs). On the other hand, statistical modelling of expression patterns with various CREs would be still very hard, mainly because of their complexity in combinations and ambiguity in recognition patterns. In this study, by applying "explainable" deep learning frameworks, or so called "X-AIs", on CREs in the whole gene promoters, we aimed to predict and model expression behaviors in the kiwifruit ripening process. A VGG-like one-dimensional convolutional neural network (1d-CNN) model was trained with the whole genes' CREs arrays constituted of 370 transcription factor (TF)-binding channels, as the explanatory variables, and their expression changes with ethylene treatment, as the response variables. We could construct a model fairly predicting ethylene-induced gene expression activation (ROC-AUC value = 0.65, at maximum). Guided backpropagation (or an X-AI technique) of the trained model successfully visualized CREs and the nucleotide residues critical to the objective expression pattern in each gene. Unexpectedly, the highest relevance for the expression prediction was identified in some novel TF-binding CREs that have not been reported for fruit ripening, suggesting kiwifruit-specific regulatory networks. Their actual contributions were experimentally validated on some representative fruit-ripening genes, with transient reporter assays with *Nicotiana benthamiana*. This X-AI-based cis -decoding framework would not only contribute to the understanding of kiwifruit-specific cis - trans regulatory networks but also provide a flexible tool for allele design to grant optimized expression in kiwifruit, via genome editing of cis - regions.

**\*Young mind award**



## High-quality genome research has significantly contributed to the domestication and exploration of kiwifruit in China

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The *Actinidia* genus comprises 54 species, 96% of which are native to the mountains of China. However, the research and exploitation of wild kiwifruit resources has long been neglected due to the difficulties in investigating agronomic traits, the complexity of ploidy levels, and the high heterozygosity of the genome. Fortunately, with advancements in genomics, metabolomics, proteomics, and germplasm innovation, Chinese scientists have made remarkable progress in the study of wild kiwifruit resources in the past decade. They have accomplished the sequencing and analysis of the genomes of more than 10 *Actinidia* species/subspecies, constructing physical and genetic maps, and developing molecular markers for breeding. And, by leveraging genomic data, they have explored molecular mechanisms of key traits, such as ultra-high vitamin C content and sex differentiation. Interspecific cross-breeding also has been successfully carried out in China, leading to the promotion of new kiwifruit hybrids with high nutritional and resistance properties. The in-depth study of kiwifruit species will support and facilitate our in-depth understanding and rapid domestication of kiwifruit resources.

## Re-understanding the scientific and breeding value of native sect. *Leiocarpae*

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The genus *Actinidia*, Actinidiaceae, has undergone at least four comprehensive taxonomic revisions since its establishment in the 19th century. In these revisions, sections and species were divided, merged, split, and revised for several times. However, the section *Leiocarpae* has never been questioned as a monophyletic lineage. Other species than *A. arguta* in the section *Leiocarpae* have received very little attention and rarely used in research and breeding program. As a representative species of this section, *A. arguta* are commercially produced annually worldwide, and the planting area is in increase at hundreds hm<sup>2</sup> each year. *A. arguta* has a short domestication history, compared to *A. chinensis*, but unfolds with a great development opportunity. Restricted by habitual thinking, *A. arguta* are only seen as a supplement type to the main commercial planting *A. chinensis* (kiwifruit). Based on recent advance, we review to re-understand the scientific and breeding value of the native Sect. *Leiocarpae* from the following aspects. Distribution of the Sect. *Leiocarpae* covers most of the geographical range of almost the entire genus, and several key agronomic traits found are unique within the genus, contributing to major variation and diversity of the genus. Based on current genome sequencing and reconstruction of paleoclimate data, speciation and adaptive evolution within the Sect. *Leiocarpae* is the fundamental scientific question for the widely distributed woody climbing plant. Phylogeographic and genetic studies on the Section are also needed to determine whether their species have differential genetic responses to Pleistocene climate changes or how they developed such a large variation. Sex determination, vernalization, rapid-softening and cold resistance constitute the representative questions of this section are the key problems that must be solved for the genomic evolution and further fast breeding. The genomics study and breeding achievements of the Sect. *Leiocarpae* has been summarized.

# Resistance, quality, cultivation and breeding in *Actinidia* species

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Kiwifruit is grown in more than 30 countries, becoming one of the important horticultural crops in the world. Here, we presented recent advances of resistance, quality, cultivation and breeding in *Actinidia* species. Comparative transcriptome and metabolome analysis reveal pyruvic acid biosynthesis is an important energy source of resistant rootstocks under salt stress in *Actinidia valvata*. AvERF73/AvERF75 regulate waterlogging tolerance of *A. valvata* through hypoxia response, MVA pathway and ROS scavenging system. AaCBF- AaBam3 module enhances freezing tolerance by degrading starch in *A. arguta*. AeNHL17 may be involved in PSA resistance defence response through activation of SA signalling pathway in *A. eriantha*. With regard to fruit quality, especially for fruit color, we elucidated the mechanism of the formation of the red-peeled trait in *A. arguta* through forward genetics research and molecular function verification. In addition, we developed male-fructifying technology and high-throughput genotyping microarray. For cultivation innovation, we explored the MVCMK (Multi-Layer Vertical Cultivation Model of Kiwifruit), aiming to improve yield. In breeding, we selected and bred a series of varieties such as 'ZMKZ-1', 'ZMKZ-2', 'ZM-2', 'ZHB', 'ZLB', etc. All this scientific research aims to serve the high quality development of the kiwifruit industry.

# Innovative approaches for fast breeding and improvement of *Actinidia* species

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The genus *Actinidia* has more than 50 species displaying wide phenotypic diversity, providing a rich genetic resource for de novo domestication and continual cultivar improvement. All *Actinidia* are dioecious and deciduous woody perennial plants with a vine growth habit. Their extended juvenile period, high heterozygosity and linkage drag of undesirable traits represent some of the main barriers in breeding. Genome editing allows for targeted mutagenesis, which has opened up a new era of accelerated improvement of woody fruit crops. Previously we used CRISPR/Cas9 to mutagenize the flowering repressor CENTRORADIALIS genes CEN and CEN4 in a diploid kiwifruit *A. chinensis*. We also manipulated sex determining genes to induce hermaphroditism using expression of the FRIENDLY BOY (FrBy) transgene in a female or mutagenesis by gene editing of SHY GIRL (SyGI) in a male *A. chinensis*. In this study, we demonstrate progress towards fast breeding and improvement of another two taxonomically distant *Actinidia* species, a large-fruited diploid *A. eriantha* and a tetraploid *A. arguta* that produces high nutritious small fruit with smooth edible skin. As in kiwifruit, gene-editing of CEN and CEN4 genes resulted in consistent phenotypes of compact growth habit, precocity and constant, year-round flowering and fruiting. The ability to perform multiple rounds of crossings in a year opens a possibility to adopt advanced breeding strategies, such as repeated backcrossing, induction of hermaphroditism and chromosome doubling enabling interspecific and interploidy crosses, to edit for high value traits or transfer desired traits from wild germplasms to commercial cultivars. These approaches can accelerate domestication and development of new varieties across the genus *Actinidia*.

# Ex situ conservation of Aotearoa New Zealand's *Actinidia* sp. genetic resources

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Exports of kiwifruit (*Actinidia* genus) make a substantial contribution (\$NZ4 billion) to Aotearoa New Zealand's earning, leading to significant investment in breeding. The New Zealand Institute for Plant and Food Research Limited (PFR) supports The Kiwifruit Breeding Centre Limited (KBC) in kiwifruit breeding. Underpinning the breeding programme is a collection comprising representatives of more than 20 kiwifruit species with almost all plants maintained as field collections. Field grown germplasm faces an evolving array of biotic and abiotic threats: this, coupled with the current challenges associated with imports of kiwifruit planting material, means preservation of existing kiwifruit genetic resources is essential. A range of ex situ strategies can be complementary to field collection. This presentation will describe different conservation strategies being developed and implemented for safeguarding PFR's kiwifruit germplasm. These include seed and pollen storage and in vitro conservation of clonal material; all of which can be enhanced by cryopreservation. Optimisation of conservation techniques for specific materials, i.e., seed, pollen and clonal propagule, is critical to ensure maximum regrowth following the desired storage period. Selection of material, technique of conservation and duration of storage will depend on the purpose of a collection. As ex situ conservation comes with a large price tag, prioritisation of genotypes for conservation may be required. Genetic tools may be used to ensure the best array of genotypes, capturing necessary diversity, is retained.

# Towards fast, non-invasive, and objective quantification of the compression-induced fruit surface deformation

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Fringe projection, known also as profilometry, surface topography, or structured-illumination reflectance imaging, is a technique that allows the determination of 3D changes in an object's surface. This is achieved by illuminating the desired area with a monochromatic fringe pattern and retrieving the object topography from the shifts observed in the pattern. The fringe projection method was previously used to detect early decay signs in oranges, genotype-related surface diversity of olive fruit, or sun-exposure-related kiwifruit skin roughness. In these measurements, fringe projection generated data showed good promise of quantifying the surface defects of different fruit. Here, fringe projection was tested as a method to detect surface deformation due to mechanical compression. This type of deformation can occur during fruit picking and handling, transport, or storage. The compression-caused deformation can indicate underlying damage such as bruising or decay and affects customer perception of fruit quality. A method that assesses fruit skin deformation would assist in the quantification and study of such damage. In this work, surface deformation was induced on kiwifruit skin (*Actinidia chinensis* var. *chinensis*) with a texture analyser (TA). To test compression detection with fringe projection, image-processing analysis based on several blurring (median filter and Gaussian filter) and edge (Laplacian filter and the Canny filter) filters was developed. The compressed area was evident in the fringe projection data beyond what was observable visually. The depth and radius of the compressed area were quantified. The radius correlated better ( $R^2 = 0.47$ ) to the deformation indicated by the TA measurement. A small disparity between measurements could be caused by fruit skin recovery between the measurements. This research shows that the fringe projection allows quantification of the compression damage and provides an objective method for fruit rejection and the analysis of the main origin of the compressed fruit across the supply chain.

# A review of non-destructive methods for kiwifruit skin assessment

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The growing environment has a profound influence on fruit development. Kiwifruit skin is the first point of contact with the surrounding environment. As such features of the skin and the near-surface layers of cellular structures have the potential to provide information on intrinsic properties of the fruit, as influenced by the environment. Extending on well-established spectroscopic methods such as near infrared spectroscopy and hyperspectral imaging, in recent years, several novel optical and image-based sensors have been utilised to assess the near-surface properties of the fruit, which may be related to important attributes of quality and storability. For instance, skin topographical information such as surface roughness and lenticel morphology can be quantified using fringe projection and used as a proxy to infer fruit growing conditions. Laser induced light backscattering imaging can be used to assess textural properties in the near-surface tissue of the fruit, providing opportunities for storability prediction and disorder detection. Using optical coherence tomography, near-surface internal structures of tissue can be characterised based on 3D volumes of image data and associated with changes in quality. From all of these methods the resulting data comes in forms that usually requires advanced analytical methods for data interpretation. Due to the wide range of inherent biological variability at both individual fruit and batch levels, large amounts of samples are often required to calibrate the sensor data with useful quality parameters usually measured destructively. This talk will provide a review of the current state-of-the-art of these non-destructive methods, provide case studies to demonstrate their capability in skin assessment and quality estimation, and identify existing challenges and potential opportunities for applications in the postharvest supply chain of kiwifruit.

# Comparison of methods for rapid non-destructive and destructive measurement of dry matter, SSC, and firmness in gold kiwifruit

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The eating experience and consumer liking of kiwifruit is related to several internal properties such as dry matter content at harvest, soluble solid content (SSC), and firmness. This paper investigates various methods for making rapid measurements of these properties. The methods can be differentiated by several characteristics such as being static (stationary) or dynamic, destructive or non-destructive, and contact or non-contact. In addition, they vary in aspects such as measurement throughput rate, cost, and degree of calibration required. Non-destructive methods allow the fruit to remain intact after measurement and are essential for kiwifruit grading applications and longitudinal studies. Non-destructive, dynamic techniques at production speeds (e.g., 10 items per second) present challenges due to constraints on geometry, the limited time to acquire measurements, and the relatively low transmission of light through the fruit. Destructive methods are generally more accurate so more suitable for system calibration but are more time consuming, e.g., 8 hours dehydrating is too slow for some applications. This paper investigates several non-destructive methods based on spectroscopy, hyperspectral imaging, and acoustic methods. The advantages and disadvantages of different light paths for making spectroscopic and hyperspectral measurements are discussed including reflectance, interactance and transmission. Results for controlled comparisons between transmission and interactance spectroscopy for dry matter and SSC prediction are presented. In addition, various destructive methods are considered and approaches to improve speed and accuracy discussed. Upper bounds in performance are obtained using replicate samples and the implications for chemometric prediction discussed.



## Non-destructive internal colour measurement of 'Zes008' kiwifruit

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The kiwifruit cultivar *Actinidia chinensis* var. *chinensis* 'Zes008' exhibits variation of 'red colour' in mature fruit, particularly in the outer pericarp tissue. Currently there are no non-destructive tools to measure internal colour of 'Zes008' kiwifruit, either pre- or postharvest. As the crop size grows in the coming years, consumers are likely to demand more consistency or uniformity to influence their re-purchase. The goal of this project was to develop novel non-destructive methods for measuring internal red colour, primarily from the outer pericarp of fruit, to enable segregation of fruit into bins with similar colour profiles. We used various handheld and inline grader instruments to non-destructively measure Near-infrared Spectroscopy (NIRS) spectra from 2000 individual fruit representing 10 orchard locations. We used a destructive imaging system, KiwiScan, to capture the images of all the fruit. These fruit images were used to interrogate the NIRS spectra and to build models to predict and segregate fruit into multiple classifications. Colour was successfully predicted using multiple NIRS instruments, with partial least squares regression-based algorithms. We used three different image classification systems to test the robustness of the NIRS devices. 1) Fruit colour was subjectively scored by eye, segregating into three classes, 'red', 'green' and 'mixed'. 2) Fruit colour was objectively scored using images captured by KiwiScan, using a Hierarchical Clustering of Principal Components analysis, segregating into eight (harvest) or 10 (storage) colour classes. 3) Fruit colour was objectively scaled by Averaging Hue angle, by transcoding RGB data directly to L\*C\*h\* format of the scanned images. In all three cases, robust predictive models were produced. Our results will be reported and discussed.

## Measuring the outer pericarp colour of red-fleshed kiwifruit – new tools with an old method

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A long-established method for measuring the colour of the outer pericarp of kiwifruit has been to remove skin and flesh to a depth of ~2 mm and measure the exposed flesh with a chromameter. This method, termed here "side-paring", is well documented for yellow-fleshed fruit and is investigated here for red-fleshed kiwifruit, in which both loss of chlorophyll and presence of anthocyanins may occur. The study involved two *Actinidia chinensis* var. *chinensis* selections, with fruit sampled three times over a 6-week period immediately prior to at-harvest maturity. The outer pericarp flesh colour was first measured objectively, by the "side-paring" method, using several different colour measurement devices, including two different Minolta chromameters (one a spectrophotometer), a small, cheap, easy-to-use device called the Colour Cube (Palette, Australia), and the DA meter (Turoni, Italy). Subsequently, the fruit were cut equatorially, with the resulting internal cross-sections subjectively assessed for colour by eye. All the measurement devices, using the "side-paring" method, were able to capture the changing average colour development in the fruit populations. Except for the DA meter, they were also able to closely match the subjective cross-section assessments at the individual fruit level. The Colour Cube was the outstanding device, being very inexpensive and easy to use compared with the other devices, and yet performing just as well.

## The Soft Fruit Sensor (SFS) - a hand-held fruit firmness measuring device developed for the kiwifruit industry

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Fruit firmness is an important quality attribute for kiwifruit, as it affects consumer acceptance, supply chain optimisation and shelf life. Currently, there are a number of methods for measuring fruit firmness, but most of these methods are either destructive or time-consuming. The result is that firmness testing has limited deployment within the industry. This paper presents a hand-held fruit firmness measuring device developed for the kiwifruit industry. The device, known as the Soft Fruit Sensor (SFS) uses small mass to delicately contact the fruit and estimate its firmness using the impact response characteristics. The device is designed to be small, lightweight, and easy to use, making it ideal for a variety of use cases. The device has been evaluated in a series of experiments, and the results are presented here. The device has the potential to be a valuable tool for the kiwifruit industry, as it can be used to quickly and easily assess fruit firmness in the field as well as provide an objective measurement alternative for clearance testing.

## Developing rationalised methods to measure and interpret firmness of kiwiberries

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Firmness is an important quality attribute for breeding and commercialisation of new *Actinidia arguta* kiwiberry cultivars. A range of measurement methods were compared for their ability to assess fruit of variable size and shape, avoid bruising, measure harvest maturity, indicate storage potential, and define fruit ripeness for eating. Best practice protocols are presented for two recommended firmness measurement methods: compression force at 1 mm using a standard probe, and impact response using a Soft Fruit Sensor recently developed by Plant & Food Research. Mathematical models are presented that interpret the measured results of the recommended methods with reference to firmness values measured using alternative methods. The purpose of this presentation is to facilitate collaboration and information exchange by international kiwiberry researchers and industry communities through the promotion of standard measurement methods.

# Multi-dimensional regulation of peel color in *Actinidia arguta*

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*Actinidia arguta* is popular with consumers for its red peel owing to biosynthesis of anthocyanin during the ripening stage. However, the molecular mechanism of red peel formation remains unclear. Here, we present a multi-dimensional regulation of peel color formation via AaBEE1-AaEIN3-AaLDOX module, which was identified through BSA-seq, RNA-seq, DAP-seq combined with various physiological and biochemical results. AaLDOX was proved to be the key functional gene of anthocyanin biosynthesis in *A. arguta*. A 29-bp indel variation in the AaLDOX promoter was found to be tightly linked with the red-/green-peeled phenotype. Molecular markers developed based on this indel could effectively distinguish 122 *A. arguta* accessions in a nature population including wild, selected and cultivated varieties, with an accuracy of ~86%, providing a valuable gene resource for kiwifruit breeding.

**\*Young mind award**

## Exploration of mechanism underlying the freezing tolerance in *Actinidia arguta* by RNA-seq and metabolome

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Kiwifruit (*Actinidia* Lindl.) is considered an important fruit species worldwide. Recently, the frequent occurrence of extremely low temperatures makes kiwifruit vulnerable, which lead to a decrease in the yield or the death of the whole plant. However, the underlying mechanism of kiwifruit's freezing tolerance remains largely unexplored and unknown. To obtain further knowledge of the mechanism underlying freezing tolerance, we chosen *Actinidia arguta* KL and RB, whose freezing tolerance is higher and lower, respectively, to identify the main pathways, important metabolites and key genes under cold stress. Under (-25°C) cold stress, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway annotations showed that the flavonoid metabolic pathways were specifically upregulated in KL, which increased its ability to scavenge for reactive oxygen species (ROS). The transcriptome changes identified in KL were accompanied by the specific upregulation of a codeinone reductase gene, a chalcone isomerase gene, and an anthocyanin 5-aromatic acyltransferase gene. Nucleotides metabolism and phenolic acids metabolism pathways were specifically upregulated in RB, which indicated that RB had a higher energy metabolism and weaker dormancy ability. Since the LPCs (LysoPC), LPEs (LysoPE) and free fatty acids were accumulated simultaneously in both genotypes, these could serve as biomarkers of low temperature-induced damages. In conclusion, the results of this study demonstrated the kiwifruit can reduce the energy metabolism by reducing phenolic metabolites and enhance the antioxidant capacity by accumulating flavonoid metabolites to improve freezing tolerance during the dormant period.

## Kiwiberries selections vary in susceptibility to skin damage

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Apart from size, a key characteristic of kiwiberries that differentiates them from current commercial kiwifruit is their thin, living skins. Significant amounts of skin damage have been observed on some kiwiberries selections but not others. A bespoke automated device to replicate observed skin damage was able to differentiate selections on their susceptibility to skin damage. The incidence of skin damage generated was not affected by fruit size within a selection, was consistent during subsequent storage at 1°C between 5 to 21 days, and did not affect softening of fruit during storage, even for selections that developed significant rates of skin damage. Microstructural observations of the skin layers of representative selections indicated that resilience or susceptibility to skin damage is likely to be related to the strength and nature of the epidermis rather than the tissues below the epidermis. These findings will be useful for fruit trait phenotyping of new kiwiberries selections.

## Understanding chilling requirements in kiwiberry

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In the *Actinidia* genus, bud dormancy occurs when metabolic growth ceases in response to temperature and photoperiod changes. To release buds from this dormant state, exposure to low temperatures is often needed to meet chilling requirements, which can vary between species and cultivars. With climate change predicted to lead to warmer winters, without adequate chilling there will be a major impact on kiwifruit productivity and profitability in New Zealand. Interest in low chill-requirement cultivars has been widely explored, while the identification of low chill genetics in kiwiberry and kiwifruit show promise. To understand the response of kiwiberry to cold, two contrasting genotypes of *Actinidia macrosperma* (low chill) and *A. melanandra* (high chill), were studied. Whole bud meristem tissue was collected from two New Zealand locations and sampled after 600 h of cold temperature treatment for metabolite and RNA-seq and metabolite analysis. Global gene changes suggested strong genotypic differences were driving chill requirements. Candidate genes were selected based on the expression difference between genotypes, and found several transcription factor genes were up regulated in the low-chill genotype. Carbohydrate related genes were up regulated in response to cold, which correlates to sugar accumulation and starch degradation for dormancy release. These findings provide insights into understanding budbreak in kiwiberry and its response to cold. With this understanding, the breeding of new climate resilient cultivars for warmer environments may be provided through key genetic information.

**\*Young mind award**



## The effect of shade during reserve accumulation on 'Zesy002' and 'Hayward' kiwifruit vine performance

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Light availability is vital for plant functions, including photosynthesis, to support carbohydrate production and reserve accumulation. Poorly managed kiwifruit vines often produce excessive vegetative growth, leading to more leaf layers, denser canopies and increased shading. We investigated the impact of shading during reserve accumulation in late summer and autumn on vine phenology and growth. Two treatments were applied to *Actinidia chinensis* var. *chinensis* 'Zesy002' and *A. chinensis* var. *deliciosa* 'Hayward' kiwifruit: a control with no shading, and a shading treatment where 50% shade cloth covered the entire vine canopy from late February to the end of June over three consecutive growing seasons. The shading treatment influenced fruit growth, quality and maturation in the current season with significant reductions in fruit weight, total soluble solids content and dry matter in both cultivars. Our study showed that shading in the previous season delayed the time taken to reach 50% budbreak in 'Zesy002', with a slight delay for 'Hayward' vines. There were no consistent effects of shading on the time of flowering in either cultivar. Total budbreak, the percentage of floral shoots, the numbers of king flowers or lateral flowers per bud and king flowers per floral shoot were all significantly reduced by shading in the previous season. These effects of shading may be attributed to reduced carbon acquisition and inadequate carbohydrate partitioning to support the current season's fruit growth and accumulation of reserves to stimulate budbreak and flower development in the following spring. Additional data will be presented on carbon partitioning. Overall, shading in late summer and autumn exerted a substantial impact on vine phenology, fruit growth and quality emphasising the importance of managing canopies well during this period.

**\*Young mind award**

## Growth, carbon acquisition and partitioning in shoots of three kiwifruit cultivars

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Shoot development underpins the vegetative and reproductive potential of kiwifruit vines during the growing season, as well as acting as an important source of reserves for the following season. Here, we compare the growth, carbon acquisition and carbon partitioning within long shoots (non-terminated shoots) of three cultivars ('Hayward', 'Zesy002' and 'Hort22D') over a growing season. The growth of shoots (stem, leaves and fruit) was measured destructively, and leaf gas exchange was recorded on every second leaf of monitored shoots, at regular intervals throughout the growing season. Budbreak was earliest in 'Hort22D' vines, followed by 'Zesy002' vines nine days later and then 'Hayward' vines after a further 13 days. The length, node number and total leaf area of shoots from each cultivar increased rapidly during spring and summer, but growth slowed earlier in 'Hayward' vines and consequently shoots were shorter than in the other cultivars. The average rate of carbon acquisition in leaves increased rapidly during spring and early summer and then declined during late summer and autumn in all vines. Leaves initially had the most rapid rate of carbon accumulation; however, stem and fruit growth became more dominant pools later in the season. By the end of the growing season the stem (the following season's cane) of all cultivars contained the largest carbon pool, with over 50% dry matter content. The similar behaviour of all three cultivars emphasises the importance of shoot growth, carbon acquisition and carbon partitioning patterns in kiwifruit vines to support both the current season's production and provide reserves to support shoot growth in the following spring.

## Effect of trunk girdling over three years on vine reserves, productivity and yield in *Actinidia chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa*

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Trunk girdling is a tool commonly used to increase fruit size and dry matter in the kiwifruit industry. It can also be used to reduce flower bud rot. Girdling works by cutting the phloem connection between the shoot and root systems. This ensures that carbon fixed in the leaves remains in the shoot at the expense of the root system, thus increasing the amount of carbon supplied to the developing fruit and shoot, whilst the girdle remains open. A girdle may be applied pre-flowering to mitigate for bud rot, after fruit set for fruit size, and one to two girdles may be applied in summer to increase fruit dry matter. Trunk girdles tend to remain open for approximately 4 weeks, potentially leading to 16 weeks of open girdle in some orchards where four girdles have been used in a single season. A healthy root system with adequate carbohydrate reserves is important during late winter to drive sap flow pre-budbreak. The aim of this work was to examine the cumulative effect of girdling over 3 years on root carbohydrate reserves, vine productivity and yield. The study was carried out over 3 years using *Actinidia chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* cultivars that received three and four girdles respectively during each season compared with vines that received no girdles. The results demonstrated that, despite a reduction in winter starch reserves in the girdled vines, there were positive outcomes of girdling for vine yield and productivity over 3 years. However longer-term there may be a need to consider root reserves and root health in heavily girdled vines.

## Genome-wide analysis of NDR1/HIN1-like genes in kiwifruit and functional analysis of NHL17 homolog under biotic stresses

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Plant NDR1/HIN1-like (NHL) genes play crucial roles in response to both biotic and abiotic stresses. However, little information about NHL genes is available for the kiwifruit. Here, we performed a genome-wide identification of the NHL genes in kiwifruit (*Actinidia chinensis*) and characterized the functional roles of the key member in response to infection by *Pseudomonas syringae* pv. *actinidiae* (Psa). We identified 33 NHL genes in *A. chinensis* from the completed genome sequence. Phylogenetic analysis revealed that AeNHLs can be classified into four distinct subgroups, with each group containing generic and specific motifs. Regulatory element analysis showed that the majority of the promoter regions of the identified AeNHLs contain MeJA-responsive and defence and stress responsive elements. Expression profiling analysis revealed that AeNHL genes are expressed in all the examined tissues of kiwifruit. In addition, we found that the expression of AeNHL17 was significantly upregulated under Psa inoculation. AeNHL17-overexpressed kiwifruit plants display significantly inhibited the infection of Psa, while silence of this gene in kiwifruit reduced tolerance to this pathogen. In briefly, we identified the NHL genes in kiwifruit and demonstrated that AeNHL17 is involved in the defence response of pathogen, suggesting that the AeNHL family members play an important role in the disease resistance of kiwifruit.

## Vigour and distance along canes affects cell proportions in 'Hayward' kiwifruit canes

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Kiwifruit canes have several distinct cell layers that perform different functions including transport, structural strength, and storage. The relative proportions of each cell layer can differ between vigour types and locations on a cane and may thus affect the overall physiology and productivity of the vine. In late winter, *Actinidia chinensis* var. *chinensis* 'Hayward' canes were collected from three vigour categories (high, medium, and low) and samples were taken at fixed distances from the base of each cane. Samples were preserved in Formalin Acetic acid Alcohol solution (FAA), sectioned using a sliding microtome, stained, and imaged using a stereo microscope. Image analysis was used to measure the cross-sectional area of each cell layer (pith, peri-medullary zone (PMZ), wood, inner bark, and outer bark) in each sample, then relative proportions were calculated. The proportion of PMZ was consistent, both along the length of canes and between vigour categories. The proportion of outer bark to wood (transport capacity) was inversely related to cane vigour and increased along the length of the cane. The proportions of both pith and inner bark to the total cross-sectional area of the cane were not significantly correlated with either vigour category or length along the cane. These relative proportions of each cell layer can directly affect carbohydrate storage or transport efficacy, and further research will investigate whether differences can be correlated with floral productivity or fruit growth.

## The effects of shading and reflective mulch on plant physiology and fruit growth in *A. chinensis*

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Kiwifruit vines are characterized by large canopies, which intercept most of the radiation in the upper part. However, leaves reach photosynthetic light saturation with only a fraction of the total radiation typical of clear sunny days in Mediterranean conditions. Since *Actinidia chinensis* is still largely managed following protocols developed for *A. deliciosa*, specific studies are needed to improve light management in *A. chinensis* orchards. This study aims at analyzing the effect of three different light micro-environments: i) a higher shading level (about 32%); ii) a white reflective mulch covering the orchard inter-row; iii) the commercial light management characterized by an anti-hail net 18% shading (control). The trial was carried out on the cv. "Jinyan" (*A. chinensis* x *A. eriantha*) in 2023. Light influence on vine physiological parameters, such as leaf gas exchanges as well as water potentials were assessed. Fruit growth, its vascular flows as well as dry matter content were also monitored during the season. Reflective mulch enhanced the diffused light within the canopy, leading to a higher fruit transpiration. Preliminary results showed slightly higher values of phloem flows to the fruit with reflective mulch during cell division. While dry matter gaining was lower in higher shading conditions. Therefore, fruit growth was influenced by the light environment. This study investigated the potential for further improvements in kiwifruit canopy light management, aimed at improving fruit growth and quality, specifically for *A. chinensis*.

**\*Young mind award**

# Boy meets Girl, everywhere? : mysteries in neo-sex chromosome evolution in kiwifruit

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Chromosomal sex determination systems in plants have evolved independently in many lineages, which can provide a powerful comparative framework to study plant-specific plasticity in sex expression. We have identified the Y-encoded two sex determinants, named Shy Girl ( SyGI ) and Friendly Boy ( FrBy ), acting as male-promoting and female-suppressing factors, respectively. Although these two Y-factors are well explainable for the dioecy widely in the genus *Actinidia*, except minor hermaphroditic mutants, recent chromosome-scale genome sequences of male accessions in *A. chinensis*, *A. rufa*, *A. polygama*, and *A. arguta*, uncovered recurrent sex chromosome turnovers in multiple lineages. We found that SyGI and FrBy were always co-ordinately transferred to other chromosomes from the putatively ancestral sex chromosome, Chr. 25, with dynamic genome rearrangements in the male-specific region of Y-chromosome (MSY). The evolution of neo-MSYs were driven via rapid bursts of independent transposable elements. Surprisingly, in any MSYs, SyGI and FrBy were physically distant (up to 700-kb) and their orders and directions were not conserved. Here, we newly conducted a series of ChIP-Seq analyses for chromatin remodelling, to discuss the following topics: (i) what mechanism(s) allowed coordinated translocation of SyGI and FrBy ?, (ii) is there any commonalities in genomic contexts amongst the neo-Y chromosomes? (iii) What is the evolutionary significance of forming these neo-Y chromosomes?

## Anaerobic metabolism and controlled atmosphere storage of 'Hayward' kiwifruit

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Controlled atmosphere (CA) storage is used commercially to prolong the postharvest life of kiwifruit, although there is always the risk of turning the fruit anaerobic. In this study, the effects of O<sub>2</sub> and CO<sub>2</sub> concentrations on the induction and re-metabolism of the anaerobic metabolites ethanol and acetaldehyde (AA) in *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit at 0°C were investigated. Production of ethanol and AA was induced only when under 0.1% O<sub>2</sub> or 20% CO<sub>2</sub>. The patterns of ethanol and AA accumulation differed, both at low O<sub>2</sub> and at high CO<sub>2</sub>. Under low O<sub>2</sub>, AA reached a maximum after 2 d, whereas ethanol continued to increase for up to 12 d. Under high CO<sub>2</sub>, both AA and ethanol increased continuously up to 12 d. The capacity to re-metabolise ethanol and AA was affected by the degree of induction and the O<sub>2</sub> and CO<sub>2</sub> concentrations post-induction. In addition, the effects were investigated of 0.1% O<sub>2</sub> for 3 or 7 days after 4 weeks of a total 16 weeks of CA (2% O<sub>2</sub> /5% CO<sub>2</sub>) storage on 'Hayward' fruit softening. While the application of anaerobic conditions mid-CA storage induced measurable amounts of both ethanol and AA, there was no effect on fruit softening. These findings suggest that for periods up to 12 d at storage temperatures, ethanol and AA are induced only by very low (0.1%) O<sub>2</sub> or very high (20%) CO<sub>2</sub> concentrations, well outside those atmospheres commonly used for CA. Also, 'Hayward' kiwifruit may withstand a short period of anaerobic metabolism mid-CA without negative fruit softening responses.



# The importance and challenge of temperature control in the kiwifruit supply chain

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Temperature is the most potent tool in the postharvest preservation of kiwifruit. Lowering temperature assists in the maintenance of kiwifruit firmness in particular. However, kiwifruit also has the potential to develop chilling injuries at low temperatures. Alternatively, some fruit require proactive ripening and colour management which can be assisted by warmer temperatures. Meanwhile, water loss, exacerbating shrivel development and ethylene production, stimulating all ripening processes, can be triggered with poor temperature management. The consequence of these combined potential impacts of temperature is the need to carefully consider the management and application of temperature control along the kiwifruit supply chain, in order to ensure that high quality fruit is delivered to the consumer. In this paper, a review of the current knowledge of methods and implications of temperature control, with emphasis on curing, conditioning, cooling, and the storage and transport environments will be discussed, with conclusions including areas of potential need for focus and future development identified.

# A review of technologies to maintain kiwifruit quality after controlled atmosphere storage

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New Zealand is a significant kiwifruit exporting country but is geographically isolated. Controlled atmospheres (CA) can extend the storage life of kiwifruit. However, CA is prohibitively expensive to implement through the whole supply chain, especially during transport. Onshore CA storage prior to shipping may extend the marketing window, but the benefit of CA storage soon declines after kiwifruit are returned to regular air storage. Post-CA, quality decline including the resumption of rapid softening occurs. This creates a particular challenge in long distance export scenarios where the transport and marketing phase of the supply chain may be numerous weeks. Hence to maintain kiwifruit quality during the post-CA period, e.g. in transport, distribution and retail, additional other technologies may be helpful. This paper reviews alternative temperature, atmosphere and ethylene management technologies that may be applied to kiwifruit during long distance transport for the purposes of maintaining the benefit of CA storage (utilised on-shore) throughout the remainder of the supply chain. Maintaining optimal refrigerated storage temperatures is a critical part of post-CA quality loss. Modified atmosphere packaging (MAP), despite the passively controlled gas composition, has potential for use during refrigerated transport and provides protection from contaminating ethylene in the external environment. Within each package, management of ethylene accumulation may also be required using either a scrubbing solution, or an ethylene action inhibitor (e.g. 1-MCP). Additionally, with care, MAP may be useful at ambient temperatures for shelf life extension. Integrating various other technologies in the post-CA supply chain may extend the value of CA storage in maintaining the quality of kiwifruit throughout the supply chain.

**\*Young mind award**

## Dynamic controlled atmosphere storage of kiwifruit

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Controlled atmosphere (CA) storage has been a feature of the kiwifruit postharvest environment since the 1980s. Traditionally, it has relied on the application of empirically determined oxygen and carbon dioxide concentrations to establish a static atmosphere which is monitored and maintained. More recently, dynamic approaches have been applied to a range of crops, mainly apples, to match the oxygen concentration to the crop, either based on respiratory metabolism or a chlorophyll fluorescence sensor. This is termed dynamic CA (DCA) storage. The use of Isolcell fluorescence interactive response monitor (F.I.R.M.) sensors has been well documented to determine the lowest safe oxygen point to set a CA. The same sensors can also detect the fruit response to changes in carbon dioxide and temperature. *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit have been investigated for the fluorescence response to low oxygen, elevated carbon dioxide and temperature changes. In addition, the beneficial effect of DCA on fruit storage has been quantified.

## Study of the dynamic of cultural microbiome of kiwifruit along post-harvest storage for the selection of beneficial microorganisms

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*A. chinensis* var. *chinensis* fruit were sampled in a commercial orchard in Italy (Latina, LT, Italy) and analysed at harvest for the cultural population of fungi, yeasts and bacteria. Approximately 250 fungal and yeast and 500 bacterial strains were identified. Fungal strains were dissected for their potential pathogenic activity on fruit. Bacterial strains were also characterized for their potential beneficial functions (e.g., IAA, production, acetoin, siderophore, ACC-deaminase) and their ability to grow at low temperatures. Sampling was performed also during the first 120 days of storage with the aim to identify the fungal and bacterial species specifically selected by cold storage. Eight bacterial species were selected for post-harvest treatments on fruit to assess their potential ability to prolong storage, reduce losses or increase fruit quality. Different strains showed the potential to inhibit *Botrytis cinerea* growth. Furthermore, six strains were able to increase sugar content in fruit, however the effect was related to an increase in softening suggesting that these strains were inducing an earlier ripening. Interesting, the inoculation generally increases storage breakdown suggesting a possible microbial component of this physiological disorder.

## Preharvest application of ozone in *Actinidia chinensis* var. *chinensis* to control post-harvest losses caused by *Botrytis cinerea*

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Kiwifruit can be stored in cold conditions for several months without losing sensorial and nutraceutical quality. However, post-harvest diseases, such as grey mould (*Botrytis cinerea*) can cause severe economical damages. Moreover, *B. cinerea* also causes premature ripening of the healthy fruit by producing ethylene and inducing its biosynthesis in infected fruit, thus amplifying its economical damages. Current control strategies rely on the use of fungicides during blooming and close to harvest to reduce primary *B. cinerea* inoculum. Nonetheless, fungicide application often results in an inadequate control due to pathogen resistance to fungicides and to the fact that they are applied prior the harvest wound, which is the main infection site. in Moreover, fungicide use in these phenological phases is particularly risk both for the potential residues on fruit and the off target effects on a beneficial insects. All these factors are fostering the research on to synthetic fungicides. The present study investigated the use of pre-harvest application of ozone and ozone emulsified oil to control post-harvest incidence of grey mould. Application of O<sub>3</sub> at 4 ppm at blooming time reduced natural population of *B. cinerea* and *Pseudomonas syringae* pv. *actinidiae* on flowers up to 97% in the first 6 days. Concerning post-harvest, ozone application reduced up to 80% grey mould incidence in comparison to control. Finally, the ozone treatment had not negative effect on any of the fruit quality parameters.

**\*Young mind award**

## Investigation into the transcriptional regulation of stigma death in *Actinidia chinensis*

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Gold kiwifruit (*Actinidia chinensis*) is a key export for New Zealand. With changing climate this crop faces future challenges. One of these challenges may be reduced natural pollination, as changing spring weather will alter flowering time and pollinator behaviour. Pollination is a limiting factor for kiwifruit yield, as the number of successful pollination events has a direct influence on fruit weight. Artificial pollination is used to supplement natural pollination by bees; this process requires larger volumes of pollen and more resources to achieve the same yield. For pollination to be successful, pollination must occur during the effective pollination period (EPP). In *A. chinensis*, the EPP is defined by programmed cell death (PCD) of the stigma. In *Arabidopsis thaliana* two NAC transcription factors, KIRA1 (KIR1) and ORESARA1 (ORE1), were identified as key regulators of stigma senescence, KIR1 appearing to play a more essential role. In *A. chinensis* we selected a collection of NAC transcription factors to study, based on expression patterns and homology to ORE1 and KIR1. Through this research, we have investigated this collection of genes to evaluate if they play a role in *A. chinensis* stigma PCD. The most promising candidate is a KIR1 homologue, NAC130 (Acc14560.1). The results of this research will provide insight into regulation of the *A. chinensis* EPP, which can then be used to optimise pollination and maximise yield. It will also expose if regulation of stigma death is a conserved model across distantly related plants.

**\*Young mind award**

# Mutagenesis of *AcCLV1* genes in kiwifruit: implications for shoot apical meristem and fruit development

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The WUSCHEL / CLAVATA (WUS / CLV) pathway has an essential role in shoot apical meristem (SAM) maintenance and floral organ development in many plant species. Mutations in genes within this pathway can induce enlarged meristems, leading to flowers with extra organs and larger fruit. However, limited information is available regarding the involvement of this pathway in woody perennial fruit species such as kiwifruit. Therefore, we aimed to identify and characterize CLV1 -like genes in kiwifruit. Two genes, *AcCLV1.1* \_Acc11900 and *AcCLV1.5* \_Acc06780, were identified, which encode leucine-rich repeat receptor-like kinases. These genes share significant homology with *Arabidopsis* and tomato CLV1 genes. Expression analysis revealed the presence of their transcripts in kiwifruit flower buds and young ovaries/fruit, suggesting their potential involvement in fruit development processes. Using CRISPR/Cas9 genome editing, we generated lines with mutations in these two *AcCLV1* genes. Mutagenesis resulted in a fasciated SAM, characterized by fasciated shoots and an increased number of petals and locules. Consequently, a significant change in floral number and, notably, larger fruit than the control were observed. Mutagenesis of *AcCLV1.1* led to more prominent phenotypes with higher yielding, larger fruit than the *AcCLV1.5* mutants. The results suggest that *AcCLV1* genes act as negative regulators, influencing the number of floral organs and fruit size of kiwifruit. These findings provide valuable insights into the genetic mechanisms governing fruit development, and contribute to our understanding of fruit size and fruit yield in kiwifruit cultivars.

## The role of LEAFY genes in floral formation in kiwifruit

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Flower development heralds sexual reproduction and ensuing production of fruits and seeds, thus optimised flowering time and flower development is crucial for productivity. In the reference plant, *Arabidopsis thaliana*, the LEAFY (LFY) gene encodes a pioneer transcription factor that plays a major role in inflorescence meristem identity and floral development. *Lfy* mutants form inflorescence shoots in the place of flowers and produced sterile flowers without petals and stamens. *LFY* shows elevated expression in the shoot apex during the transition to flowering, while the TFL1 / CEN flowering repressor restricts *LFY* expression to the flanks of the shoot apical meristem, enabling indeterminate growth of the primary inflorescence. In this study, the expression and function of two kiwifruit LFY genes were analysed. Both *AcLFY* genes were predominantly expressed in terminal and axillary buds at similar levels during spring and summer when the initial stages of kiwifruit floral evocation occur. In addition, they were regulated by the floral repressors CEN/CEN4 in axillary buds, further implicating them in flowering. Over-expression of *AcLFY1* and *AcLFY2* induced early flowering and solitary flowers instead of secondary shoots in *Arabidopsis*. *AcLFY* over-expression in kiwifruit is underway. *AcLFY* genes were also edited in the fast-flowering *cen / cen4* background. The flowers in *Aclyf1Aclyf2* biallelic-edited lines had the most extreme phenotypes. They often aborted early in development or exhibited enlarged floral organs with abnormal morphology. The flowers were infertile and failed to produce fruit. This study implicates the two *LFY* genes being critical for normal floral and fruit development in kiwifruit. To further understand the mechanism of LFY action, the interactions between LFY and other floral regulators such as AP1, AP3, and AG are being investigated through promoter activation transient assays.



## Characterization of *Actinidia chinensis* FD genes and their role in kiwifruit flowering

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Arabidopsis FD gene homologs encode bZIP transcription factors that interact with FLOWERING LOCUS T (FT) proteins to induce flowering in many plant species. In woody perennial plants such as kiwifruit, (*Actinidia* spp.) the function of FD genes is largely unknown. In this study, we isolated three closely related *A. chinensis* FD homologs, AcFD, AcFD1 and AcFD2. We investigated their function by analysing their expression profiles, examining their interactions with different FT proteins, and observing their transgenic phenotypes. AcFD was mainly expressed in actively growing buds in summer, while both AcFD1 and AcFD2 demonstrated highest expressions in dormant buds through autumn and winter. Using yeast-two hybrid experiments, we revealed that each of the three *A. chinensis* FT proteins interacted with AcFDs with differing interaction intensities. Ectopic expression of AcFD and AcFD2, and their phosphorylated counterparts, resulted in early flowering in Arabidopsis but not in kiwifruit. Using CRISPR-Cas9, we produced *fd* mutants and *fd1-fd2* double mutants in fast-flowering kiwifruit, which did not demonstrate a delay in flowering time. Overall, these data suggest the AcFDs are the functional homologs of Arabidopsis FD, which may interact with different AcFT proteins to regulate flowering, but the mechanism in kiwifruit may be more complex.

## The fate of inflorescence meristems during their transition to inflorescences at anthesis

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Kiwifruit inflorescences, with a terminal flower and two lateral flowers, can develop from undifferentiated meristems in dormant buds of most commercial *Actinidia chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* cultivars. During floral differentiation in spring, the fate of the inflorescence meristems can vary from failure to begin, abortion of partially formed terminal or lateral flowers, to complete formation of an inflorescence at anthesis. This extended period of floral differentiation in spring determines the potential number, size, and shape of fruit on a vine. In a preliminary investigation of floral development, we monitored the development of inflorescences within buds and developing shoots of *A. chinensis* var. *chinensis* 'Zesy002', 'Zes008' and *A. chinensis* var. *deliciosa* 'Hayward' kiwifruit. In spring, during the initial stages of budswell and budbreak, about 50% of meristems either failed to begin or ceased inflorescence development. Inflorescence numbers then remained constant until the later stages of rapid shoot growth, prior to anthesis, when another 10–30% of inflorescences aborted on shoots. Failure to develop was higher in lateral flowers compared with terminal flowers and in inflorescences at basal positions on shoots. Flower ovary size and shape also varied within shoots. This study underpins understanding of the processes controlling floral differentiation and will contribute to the successful breeding and management of kiwifruit cultivars.

## Effect of biostimulant applications with UAV to avoid abiotic stress conditions

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In Chile, kiwi orchards present abiotic stress conditions during the summer period due to high radiation and temperature. For this reason, this work aimed to evaluate the effect of biostimulant applications with UAV to avoid abiotic stress conditions. The study was carried out in the 2022-23 season in a kiwi cv Hayward orchard, located in the commune of Sagrada Familia, Chile. The individual effect of 2 biostimulants based on vegetable amino acids was evaluated versus a control treatment. Measurements of the water status of the plants were made through the water potential stem and the leaf temperature obtained by a portable infrared radiometer and a thermal camera equipped in a UAV. The results show that both biostimulants cause a statistically significant decrease in leaf temperature in comparison to the control treatment. This decrease was between 1.0 to 1.5°C. In conclusion, it can be pointed out that biostimulants applied with UAV can reduce the risk of thermal stress in a kiwi orchard.

## Establishment, in vitro multiplication and acclimatization of *Actinidia chinensis* adapted to tropical highlands

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Kiwifruit is a new fruit crop in the tropical highlands of Mexico. The massive propagation of selected kiwifruit to establish new orchards is necessary. Thus, in vitro culture is an alternative for its propagation. This study seeks to standardize the establishment and in vitro multiplication of kiwifruit plants adapted to tropical highlands for subsequent transplanting to the field. Disinfectant treatments and tissue regeneration for petiole, pedicel, and buds were evaluated, as well as disinfectant treatments and establishment medium formulations for seeds. In addition, four formulations of MS growing medium were evaluated for plant multiplication, followed by the evaluation of survival during acclimatization. All trials corresponded to a completely randomized design with at least 23 replicates. Data were analyzed by normality tests and parametric and non-parametric analyses using the InfoStat statistical software. The results indicated that the best treatment for the generation of new plants was from seeds disinfected with 70% ethyl alcohol for 10 minutes together with 30% Clorox® commercial bleach for 20 minutes and VW establishment medium. MS medium supplemented with 2 N ammonium nitrate favored shoot multiplication, while 50% acclimatization was possible for plants originated from seeds of adapted plants. In vitro seed establishment preserves variability, reduces development time and homogenizes germination. Nitrogen in the culture medium stimulated shoot multiplication, vegetative growth and vigor of kiwifruit plants.

# The potential for rootstocks to devigorate kiwifruit polliniser scions

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Kiwifruit pollinisers can occupy between 2% and 10% of an orchard's area. While required to produce a crop, the loss of orchard production area to these plants represents a loss in potential productivity. In addition, excessive canopy vigour of pollinisers creates shade, reducing the floral fertility of adjacent vines and requiring labour intensive management practices in both summer and winter. Dwarfing rootstocks could provide a means to reduce the canopy size of kiwifruit polliniser scions, reducing the area they occupy in the orchard and the need for extensive shoot management. We assessed polliniser scion vigour and progression of flowering during the seventh season of growth for the male cultivars 'Bruce' and 'Sparkler' grafted to 26 different rootstocks. Scion vigour, assessed as trunk cross-sectional area ranged from 3.3 to 24.8 cm<sup>2</sup>, demonstrating the potential of rootstocks to devigorate polliniser scions. Scion trunk cross-sectional area was found to be an adequate predictor of estimated canopy volume. Small differences in the date of 50% flowering in 'Sparkler' were found between some of the rootstocks, with up to five days' difference between the earliest and latest flowering. This demonstrated potential of rootstocks to devigorate kiwifruit polliniser scion vigour would allow the development of new training systems, enabling substantially reduced canopy area and requiring less canopy management, without reducing polliniser effectiveness.

# Research and application of traditional Chinese medicine agricultural techniques in kiwifruit

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Traditional Chinese Medicine (TCM) agricultural technology is the application of TCM theory to agriculture, combining botanical extracts to form biological pesticides and preparations instead of fertilizer and chemical pesticides, to achieve green and organic production of agricultural products within a healthy ecological environment. Fruit safety is becoming more and more prominent in food production, according to the principles and methods of TCM agriculture, a preliminary study and application have been conducted in kiwifruit, and significant results have been obtained in the control of soil heavy metals, integrated management of pests and diseases, and fruit postharvest storage and preservation. The application of plant extracts can improve the physical and chemical properties of soil. Reduce the content of total cadmium and available cadmium in soil, and reduce the absorption of cadmium by plants. Effective targeting inhibits the growth of pathogens such as *Pseudomonas syringae*, *Phyllosticta capitalensis*, *Corynespora cassiicola*, and *Pseudomonas viridiflava*. The extracts of *Scutellaria baicalensis*, *Syzygium aromaticum*, *Acorus tatarinowii*, and *Pogostemon cablin* had a 95.45% cure rate for the spot caused by kiwifruit bacterial canker. More than twenty kinds of antimicrobial agents were obtained from extracts of traditional Chinese herbs, which have a strong inhibitory effect on the fungi of the *Diaporthe phaseolorum* and *Botryosphaeria dothidea*. The compound preservative developed can significantly reduce the softening of the 'Hong Yang' fruit. TCM agricultural technology is effective in healthy cultivation, and safe and organic production, and provides new ideas for sustainable green development of kiwifruit.

## Towards sustainable and climate resilient yield in kiwifruit

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Climate change will drive more extreme variability in kiwifruit flower numbers, and current mitigation techniques (e.g. labour and chemicals) may become highly unsustainable. Therefore, achieving profitable and sustainable crop yields will become increasingly challenging in the future. Flower initiation is well studied and characterised from a biological and molecular point of view, whilst flower differentiation and its effect on flower numbers has received less attention. There is a large variation in flower numbers per inflorescence across *Actinidia* species. We have recently identified some near-isogenic lines, within our glasshouse material, with contrasting flower numbers per inflorescence phenotypes. We have characterised floral buds at the phenotypic, metabolic, and molecular level to identify key determinants of flower numbers during inflorescence differentiation. We believe new knowledge on how different metabolites interact to control flower number will underpin the development of novel, sustainable tools to manipulate flower abortion/retention and influence flower quality.

