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PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**CONSENSUS DOCUMENT ON THE BIOLOGY OF APPLE
(*Malus domestica* Borkh.)**

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No. 66

Consensus Document on the Biology of Apple
(*Malus domestica* Borkh.)

Environment Directorate

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**OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France**

E-mail: ehscont@oecd.org

FOREWORD

The consensus documents prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology (WG-HROB) contain information for use during the regulatory assessment of the environmental safety (or ‘biosafety’) of a particular product. In the area of plants, these are being published on information on the biology of certain species of crops and trees, selected traits that may be introduced into plant species, and biosafety issues arising from certain general types of modifications made to plants.

This document addresses the biology of apple (*Malus domestica* Borkh.).

Belgium and Germany served as the co-leads in the preparation of this document, and the draft has been revised based on the input from other member countries and stakeholders.

The WG-HROB endorsed this document, which is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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SECTION 1. SPECIES OR TAXONOMIC GROUP

1.1. Classification and nomenclature

The genus *Malus* belongs to the rose family (Rosaceae) which is traditionally divided into four subfamilies on the basis of fruit type. These include: Rosoideae (e.g., *Rosa*, *Fragaria*, *Potentilla* and *Rubus*; fruit, achene); Prunoideae (e.g., *Prunus*; fruit, drupe); Spiraeoideae (e.g., *Spiraea*; fruit, follicle or capsule), and Maloideae (e.g., *Malus*, *Pyrus* and *Cotoneaster*; fruit, pome) (Schulze-Menz, 1964). The systematic classification of Rosaceae has changed over the years and molecular analysis has added to the debate on the subfamily groupings (Potter et al., 2007). Using nucleotide sequence data from nuclear and chloroplast regions of 88 genera of Rosaceae, the Rosaceae family was re-classified into three subfamilies: Rosoideae (base chromosome number $x =$ mostly 7), Dryadoideae (e.g., *Cercocarpus*, *Dryas* and *Purshia*; fruit, achene or aggregate of achenes; $x = 8$ or higher) and Spiraeoideae (mostly $x = 8, 9$, and rarely $x = 15$ or 17). All genera previously assigned to Prunoideae ($x = 8$) and Maloideae ($x = 17$) were included into the Spiraeoideae (Potter et al., 2007). This subfamily, however, is to be called Amygdaloideae rather than Spiraeoideae under the International Code of Nomenclature (McNeill et al., 2012). The latest classification of the Rosaceae family is thus based on three subfamilies Rosoideae, Amygdaloideae, including *Malus*, and Dryadoideae. Although the traditional definition of the four major rosaceous subfamilies may be collapsing from a taxonomic view, this grouping still has great utility from an economic and horticultural standpoint, and is still commonly used in literature.

Figure 1. Classification and synonyms of *Malus domestica*

<u>Scientific name:</u>	<i>Malus domestica</i> Borkh.	
<u>Pertinent synonym(s):</u>	<i>Malus malus</i> (L.) Britton, nom. inval.; <i>Malus pumila</i> auct.; <i>Malus pumila</i> var. <i>domestica</i> (Borkh.) C. K. Schneider; <i>Malus sylvestris</i> auct.; <i>Malus sylvestris</i> var. <i>domestica</i> (Borkh.) Mansf.; <i>Pyrus malus</i> L.	
<u>Taxonomic context:</u>	Family	Rosaceae Juss.
	Subfamily	Amygdaloideae
	Tribe	Maleae
	Subtribe	Malinae
	Genus	<i>Malus</i> Mill.
	Section	<i>Malus</i>
	Species	<i>Malus domestica</i> Borkh.

Source: USDA-ARS (2018).

The genus *Malus* is currently organised into six taxonomic sections, one being *Malus* to which the species *Malus domestica* belongs (USDA-ARS, 2018; see Figure 1). Altogether, 59 species of *Malus* are cited in the taxonomy database of the USDA-ARS Germplasm Resources Information Networks (GRIN) and are provided in Annex A. However, the number of species included in the genus is an ongoing subject of debate, revolving around the acceptance of putative hybrids. For example, *M. arnoldiana* (*M. baccata* × *M. floribunda*), which is considered a ‘secondary species’ developed through interspecific

hybridisation of ‘primary species’ (Jackson, 2003; Luby, 2003; Rieger, 2006; Hancock et al., 2008).

The cultivated apple *M. domestica* is thought to be the result of interspecific hybridisation (see 1.3.1). The binomial *M. domestica* Borkh. has been generally accepted as the appropriate scientific name replacing the earlier scientific name *M. pumila* (Korban and Skirvin, 1984; Qian et al., 2010). Throughout its history of cultivation, more than ten thousand cultivars of *M. domestica* have been developed, although many of those are now lost (Way et al., 1990; Janick et al., 1996; Rieger, 2006). Currently, about 100 cultivars are grown commercially, the most popular worldwide including: ‘Fuji’, ‘Delicious’, ‘Golden Delicious’, ‘Gala’, ‘Granny Smith’, ‘Idared’, ‘Jonagold’, ‘Braeburn’, ‘Cripps Pink’, ‘Jonathan’, ‘Elstar’ and ‘McIntosh’ (Jackson, 2003; see Figure 2). Most of the cultivars are diploid, while some of them are triploid (e.g., ‘Jonagold’, ‘Mutsu’, ‘Schöner von Boskoop’) and a few are tetraploid (e.g., ‘Antonovka Ploskaya’, ‘Wealthy Tetraploidnyi’, ‘Papirovska Tetraploidnaya’, ‘McIntosh Tetraploidnyi’).

Figure 2. Commercially grown apple cultivars



Note: (a) ‘Fuji’, (b) ‘Delicious’, (c) ‘Golden Delicious’, (d) ‘Gala’, (e) ‘Granny Smith’, (f) ‘Idared’, (g) ‘Jonagold’, (h) ‘Braeburn’, (i) ‘Cripps Pink’, (j) ‘Jonathan’, (k) ‘Elstar’ and (l) ‘McIntosh’.

Source: Courtesy of Bundessortenamt and Julius Kühn-Institute, Germany.

1.2. Description

M. domestica is botanically described as follows:

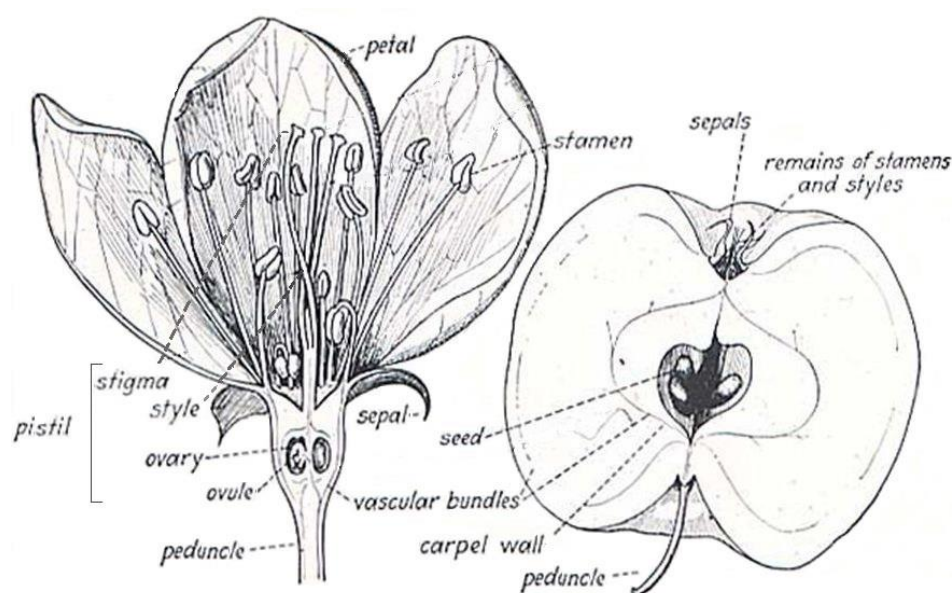
Tree – a small- to medium-sized, branched, deciduous tree with a single trunk and a broadly spreading canopy. The trees are generally 2-10 m tall (in cultivation, tree size and shape are heavily dependent on rootstock and training (planting) system). Young stems and twigs are somewhat tomentose (hairy), while older branches are glabrous (smooth) (Bailey and Bailey, 1976; Webster, 2005a). Spurs (very short shoots) grow very slowly and primarily produce flowers and subsequent fruits. They are formed on one-year old shoots. Root suckers can emerge from the rootstock.

Leaves - are alternately arranged, dark green, simple oval-shaped with a serrated edge, 4-13 cm long x 3-7 cm wide, with irregularly saw-toothed margins, and usually hairy beneath (Webster, 2005a; Rieger, 2006).

Buds - are purplish brown, ovoid and densely hairy. Buds either give rise to shoots/leaves (vegetative buds) or to flowers (flower buds). Flower buds are larger and more plump than growth buds and have a downy surface.

Flowers - are 3-4 cm in diameter. Each flower blossom, has five sepals, five petals varying from white to pink, and about 20 stamens with yellow anthers in three whorls (10+5+5). The pistil comprises of a stigma and five styles united at the base (Jackson, 2003; Hancock et al., 2008). The five styles are slightly longer than the stamen. The ovary is inferior, positioned beneath the sepals, petals and stamen. The peduncle and calyx (all sepals) are usually woolly, and the calyx is persistent in the fruit (Webster, 2005a). Flowers are usually terminal on spurs, although they may grow laterally from one year old shoots in some cultivars, borne in groups of 4-6, in inflorescences that have variously been described as corymbs, corymbose racemes, cymes, and false cymes (Jackson, 2003; Rieger, 2006).

Figure 3. Flower and fruit of apple, cut lengthwise showing relation of the parts of the flower



Source: adapted from Iowa State University (2003).

Fruit - is an ellipsoid to obovoid, globe-like pome indented at the base and at the apex (see Figure 3). The fruits are usually greater than 5 cm in diameter weighing 200-350 grammes. Fruits vary in colour and can be uniformly red, green or yellow, or bi-coloured. Bi-coloured fruit can be striped or blushed red on a yellow or green background (see Figure 2). Each fruit contains a cortex of (edible) flesh between the skin and the core line. The central core has fleshy pith with a papery capsule of five fused carpels. Each carpel typically contains two seeds. Seeds are smooth, shiny, and chestnut brown (Jackson, 2003; Rieger, 2006).

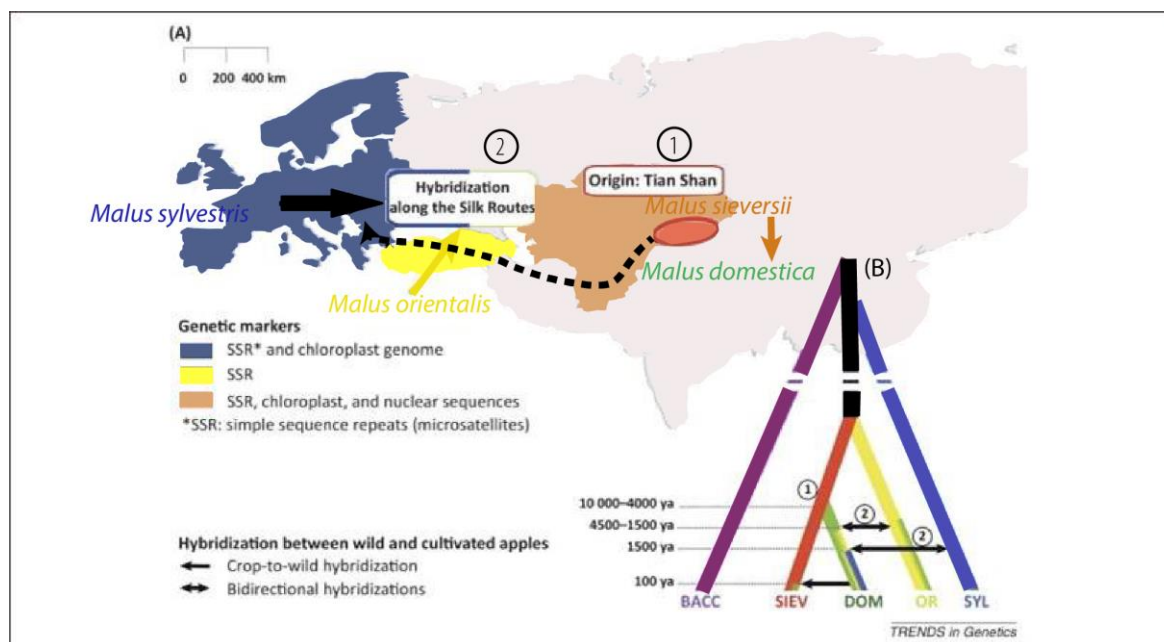
Roots - consist of a horizontal layer of permanent, thickened, spreading scaffold roots less than 50 cm from the surface, and numerous vertical ‘sinkers’ descending to an impermeable layer or water table (Jackson, 2003).

1.3. Centres of origin and diversity, geographic distribution, natural and managed ecosystems and habitats, cultivation and management practices

1.3.1. Centres of origin and diversity

The centre of origin for domesticated apple species lies within the Tian Shan forest regions of Central Asia, including Kazakhstan, Kyrgyzstan and Tajikistan (Vavilov, 1951; Dzhangaliev, 2003). The wild species *Malus sieversii* (Ledeb.) M. Roem. occurring in these forest regions has been identified as the initial progenitor to the genome of the cultivated *M. domestica* on the basis of morphological, historical and molecular evidence (Robinson et al., 2001; Harris, Robinson and Juniper, 2002; Velasco et al., 2010; Duan et al., 2017).

Figure 4. Evolutionary history of the cultivated apple



Note: (A) Origin in the Tian Shan Mountains (1) followed by dispersal (2) from Asia to Europe along the Silk Route. Arrow thickness is proportional to the genetic contribution of various wild species to the genetic makeup of *Malus domestica*. (B) Genealogical relationships between wild and cultivated apples. Approximate dates of the domestication and hybridisation events between wild and cultivated species are detailed in the legend. Abbreviations: BACC, *M. baccata*; DOM, *M. domestica*; OR, *M. orientalis*; SIEV, *M. sieversii*; SYL, *M. sylvestris*; ya, years ago.

Source: Cornille et al. (2014).

The domestication of *Malus* occurred around 8 000 to 2 000 BCE in Central Asia, possibly near Almaty, Kazakhstan (Vavilov, 1930 as referred to in Robinson et al., 2001; Zohary, Hopf and Weiss, 2012). Apple seeds and trees from selected forms were then dispersed along the trade routes of the Silk Route from Central Asia, east to People's Republic of China and west to Europe (Harris, Robinson and Juniper, 2002), resulting into random establishment of apple germplasm along the Silk Route (see Figure 4).

Hybridisations occurred between the apples coming from central Asia and closely related species present along the Silk Route. This gave rise to diverse forms of hybrids from which the present day cultivated apple might have been selected and propagated by vegetative means. Several species contributed to the genetic background of the current apple populations, some of the ones that are considered to have contributed are the Siberian crab apple *M. baccata* L. (Borkh.), the Caucasian crab apple *M. orientalis* Uglitzk., and the European crab apple *M. sylvestris* L. Mill. (Cornille et al., 2012). The wild European crab apple *M. sylvestris*, in particular, is considered to be a major contributor to *M. domestica* in Western Europe, as it is genetically more closely related to this species than to its Central Asian progenitor, *M. sieversii* (Cornille et al., 2012, 2014; Duan et al., 2017).

1.3.2. Geographic distribution

M. domestica is cultivated throughout temperate areas of the world. In general, *M. domestica* is well adapted to a range of climates, but its ideal growing conditions are the cool-temperate zone between about 35-50° latitude with high light intensity, warm days and cool nights (Webster, 2005b; Rieger, 2006). Its range is farther north than most other fruit crops due to its relatively late blooming and cold hardiness (Rieger, 2006). It is also cultivated in less suitable climates (i.e., semi-arid, subtropical and tropical) where irrigation, altitude, and various farming practices are used to overcome climatic limitations (Westwood, 1993; Hampson and Kemp, 2003). An example of the range of temperatures over which apples are successfully produced, is provided by Jackson (2003). At the extreme cold end of the range, Poland, with winter monthly minimum temperatures of -17°C and summer monthly maximum temperatures of 30°C has successful apple cultivation. Egypt with winter minimums of 1°C and summer maximums of 43°C reflects the extreme warm end of the range (Jackson, 2003). Global climate change is likely to affect the current geographic distribution of *M. domestica*.

1.3.3. Ecosystems and habitats where the species occurs natively, and where it has naturalised

M. domestica is a product of selection by human intervention and hybridisations over thousands of years in many parts of the world (see 1.3.4). Outside of its cultivation areas, *M. domestica* has naturalised in different parts of the world, where it grows in abandoned pastures, clearings, roadsides, and borders of woods (Randall, 2017).

Wild apple populations, also known as 'crab apples', are native throughout the Northern hemisphere in temperate areas (Luby, 2003). They are mainly found on the edge of woods and areas of scrub, in moist or coastal regions (e.g., Routson et al., 2012). In Europe there are five endemic *Malus* species – the crab apple from Sicily *M. crecimannoi*, the Florentine (or Hawthorn-leaf) crab apple *M. florentina*, the Paradise apple *M. pumila*, the Lebanese (or three-lobed) crab apple *M. trilobata* and the European crab apple *M. sylvestris* (IUCN, 2019). There are four *Malus* species native to North America – the Southern crab apple *M. angustifolia* (United States), the Sweet crab apple *M. coronaria*

(Canada, United States), the Oregon crab apple (also termed Pacific crab apple) *M. fusca* (United States, Canada) and the Prairie crab apple *M. ioensis* (United States) (VASCAN, 2018; USDA-NRCS, 2018). *M. sieversii* is native to Western People's Republic of China and Central Asia (Richards et al., 2008; Gharghani et al., 2009; Gross et al., 2012; Nikiforova et al., 2013; Volk et al., 2013), but has become a rare and threatened plant in People's Republic of China (Yan et al., 2008; IUCN, 2019).

Crab apple accessions of different *Malus* species may be grown as ornamentals in landscaping, parks or gardens (Fiala, 1994). Many of these accessions offer prolific spring bloom and a wide range of decorative fruits differing in size, shape and colour (Fiala, 1994). Further, crab apple may be used as pollinisers in apple orchards.

1.3.4. Intensively managed ecosystems where the species is grown or occurs on its own, including management practices

The apple *M. domestica* is one of the most widely cultivated tree fruits (Table 1). Cultivation started as early as 4 000 BCE in the Near East (Figure 4) and apples reached the eastern Mediterranean region by 2 000 BCE and Greece and Italy by 900 and 800 BCE.

Table 1. Overview of continents, territories and countries where apple is cultivated

Continent	Territories and countries
Africa:	Algeria, Egypt, Kenya, Madagascar, Morocco, La Réunion (France), South Africa, Tunisia, Zimbabwe.
Americas:	Argentina, Bolivia, Brazil, Canada, Caribbean islands, Chile, Colombia, Ecuador, Grenada, Guatemala, Honduras, Mexico, Paraguay, Peru, United States, Uruguay.
Asia:	Afghanistan, Armenia, Azerbaijan, Bhutan, People's Republic of China, Cyprus ^{1,2} , Georgia, India, Indonesia, Iran, Iraq, Israel ³ , Japan, Jordan, Kazakhstan, Korea, Kyrgyzstan, Lebanon, Myanmar, Nepal, Pakistan, Philippines, Syria, Tajikistan, Thailand, Turkey, Turkmenistan, Uzbekistan, Viet Nam, Yemen.
Europe:	Albania, Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, United Kingdom.
Oceania:	Australia, New Zealand.

Source: FAO (2019).

¹ Note by Turkey:

The information in this document with reference to "Cyprus" relates to the southern part of the Island. There is no single authority representing both Turkish and Greek Cypriot people on the Island. Turkey recognises the Turkish Republic of Northern Cyprus (TRNC). Until a lasting and equitable solution is found within the context of the United Nations, Turkey shall preserve its position concerning the "Cyprus issue".

² Note by all the European Union Member States of the OECD and the European Union:

The Republic of Cyprus is recognised by all members of the United Nations with the exception of Turkey. The information in this document relates to the area under the effective control of the Government of the Republic of Cyprus.

³ The statistical data for Israel are supplied by and under the responsibility of the relevant Israeli authorities. The use of such data by the OECD is without prejudice to the status of the Golan Heights, East Jerusalem and Israeli settlements in the West Bank under the terms of international law.

The Roman armies carried apples across Europe, planting seeds wherever they settled. By the 1200s, cultivated apples were becoming increasingly popular in the Nile Delta and throughout Europe, where they appeared in the gardens of both royalty and commoners. By the 1600s there were at least 120 cultivars of apple described (Luby, 2003). European colonists introduced *M. domestica* to the Americas (1500-1600s), South Africa (1650s), Australia (1788), and New Zealand (1814). By the late 1800s, *M. domestica* had been introduced into southern and eastern Asia, where it supplanted the Chinese soft apple, *M. x asiatica Nakai*, the primary cultivated apple in that region for over 2 000 years (Luby, 2003; Hancock et al., 2008). Today, the global apple market is dominated by European and North American *M. domestica* varieties.

Table 2. Highlights of a typical management schedule for apple production

Time of Year - Phase	Action
Winter - dormancy	<ul style="list-style-type: none"> - Prune dormant trees - Remove root suckers - Perform root pruning - Analyse soil composition - Apply soil fertiliser and lime - Apply herbicides
Spring - fruit set	<ul style="list-style-type: none"> - Perform pruning (mechanical) - Apply mulching - Perform irrigation - Apply frost protection - Place bees in orchards when blossom begins - Apply chemical and/or mechanical thinning - Monitor and control pests (insects and diseases) - Monitor and control weeds
Summer - fruit growth	<ul style="list-style-type: none"> - Perform irrigation - Apply hand thinning - Perform summer pruning - Remove root suckers - Apply mulching - Apply foliar fertiliser (calcium to prevent bitter pit) - Monitor and control pests (insects and diseases) - Monitor and control weeds - Prepare soil for planting
Fall - harvest period	<ul style="list-style-type: none"> - Harvest apples - Store apples in cold or controlled atmosphere storage - Apply leaf fall spraying - Build the trellis (support) system for new trees - Perform tree planting

Source: Adapted from AAFC (2013).

M. domestica is a labour intensive, highly managed crop, especially by the time an orchard has reached maturity and is ready for commercial production. Although about no fruit is produced during the first year(s) after plantation, there is still a number of agronomic issues that must be properly managed to ensure good tree growth. These include nutrient amendments (through fertigation or direct soil application), disease and pest control, irrigation, weed control and pruning. Table 2 highlights a typical management schedule for apple production. Trees and fruit are prone to a number of fungal, bacterial and pest problems (see 5.1.1), which can be controlled by a number of non-organic and organic

means. Many commercial orchards pursue a program of chemical sprays, but a trend in orchard management is the use of biological control methods, which include, for instance, introduction of a natural predator to reduce the population of a particular pest (see 5.1.2.2).

M. domestica is not regarded as a weed of agriculture (Randall, 2017). Volunteer plants originating from seed in apple orchards are rare due to the perennial nature of the crop and orchard management practices that include herbicide treatment of the tree row and mowing of the alley between rows (Stover and Marks, 1998).

SECTION 2. REPRODUCTIVE BIOLOGY

2.1. Generation time and duration under natural circumstances, and where grown or managed

The whole sexual reproduction cycle (fertilisation, seed formation and seedling growth) only occurs naturally in the wild (see 2.1.1), in the production of some rootstocks and in apple breeding programs (for the latter two see 3.2). The life cycle of apple in managed ecosystems starts from the adult phase (see 2.1.2).

2.1.1. *Life cycle of apple under natural conditions*

Under natural circumstances the life cycle of wild apple starts from a seed, released by frugivorous animals or by rotting of fruit during winter on the ground. After germination, the juvenile growing period follows. During this period morphological and physiological characteristics of the plant differ relative to the adult stage. The inability to flower and produce fruit and seeds is one of the main characteristics of the juvenile period. The plant enters the adult stage once flower bud differentiation occurs. During the adult stage, the plant is fertile and will flower almost every year. The juvenile period of wild apple plants under natural conditions usually takes between 6-12 years and is influenced by environmental as well as genetic factors. It can happen that years with a high crop load are followed by years with low to no crop load, a phenomenon known as biennial bearing (Tromp, Webster and Wertheim, 2005).

2.1.2. *Life cycle of apple in managed ecosystems*

In managed commercial orchards one- or two-year-old trees are planted that were propagated in fruit tree nurseries by budding or grafting on rootstocks. The buds or grafts are collected from mature commercial apple cultivars. This means that the resulting trees are no longer juvenile. Nevertheless, it is unlikely that one-year-old trees will bear fruit. In commercial orchards the first yield will be obtained from the second year on and will progressively increase until a maximum yield is reached after 5-7 years.

The life span of a commercial high-density apple orchard is about 15 years, although many orchards do maintain a sufficient production beyond this age (personal communication fruit growers). A number of factors, including tree health, pests, apple cultivar, soil quality, environmental factors (i.e., heat units, winter injury), market opportunities, etc. play a role in the life span of a commercial orchard.

Generally, apple fruits reach maturity about 120-150 days after flowering, but some cultivars mature in as little as 70 days, others in as long as 180 days (Rieger, 2006). Time to maturity varies with temperature (e.g., warmer temperatures reduce time to maturity) and is therefore geographically dependant. However, rankings of “early” or “late” maturing varieties relative to each other are fairly consistent (Jackson, 2003). Short-season cultivars tend to have a wide climatic tolerance; they do well in colder, northerly apple-producing regions such as Canada (e.g., ‘McIntosh’) and may also be grown as early-season crops in countries like New Zealand and France (e.g., ‘Cox’) (Jackson, 2003). Long-season cultivars like ‘Braeburn’, ‘Fuji’, ‘Cripps Pink’ and ‘Granny Smith’ generally cannot be grown successfully in northern areas and do best in the milder climates, mainly in the southern hemisphere (Hampson and Kemp, 2003; Jackson, 2003).

2.2 Reproduction (production of flowers, fruits, seeds and root suckers)

2.2.1. Floral biology

2.2.1.1. Flower development

In apple, flower development lasts between nine and ten months, starting with the formation of floral primordia in the mixed flower buds (i.e., buds producing flowers in addition to leaves and shoots) in summer and early autumn. At leaf fall, flower and leaf primordia are present in a large portion of the flower buds (Kotoda et al., 2000; Dennis, 2003; Jackson, 2003; Koutinas, Pepelyankov and Lichev, 2010). During winter development slows due to bud inhibition by (endo)dormancy. Bud break follows after winter-chilling and heat-unit requirements are met. The king (apical) flower of the apple inflorescence opens first, followed by the lateral flowers (Jackson, 2003). Flowering occurs in spring when white to deep pink flowers develop in a cyme-like inflorescence of 4-6 flowers (Figure 5). Apple flowers can be borne in both terminal and lateral flower buds on both spurs and shoots. Flower clusters on lateral buds open later than do those on terminal buds and generally produce smaller fruit (Jackson, 2003). The flowering period takes 1-4 weeks, depending on the weather conditions, beginning with the (king flower of the) terminal flower buds and ending with the (lateral flowers of the) lateral flower buds.

Flowering is affected by many biotic (endogenous phytohormones, previous year's crop load, pathogens and pests) and abiotic (light, water stress, nutrients, temperature and exogenously applied chemicals) factors. Also cultivation practices drive flowering and include: grafting, pruning, scoring and/or ringing the base of the tree (Jackson, 2003). The flowering period can significantly differ between cultivars. The difference in flowering time between commercial cultivars and local cultivars can be more than one month in colder temperate regions (e.g., North West Europe) and about 2-3 weeks in warmer regions (e.g., South Europe).

Figure 17458. Open king flower and closed lateral flowers



Source: Courtesy of A. De Schrijver.

2.2.1.2. *Effective pollination period*

The length of the flowering period during which viable pollen is produced varies depending on weather conditions and generally lasts from 7 to 30 days (Jackson, 2003). During flowering, the stigma produces extracellular secretions which provide a moist environment for pollen deposition and germination (Jackson, 2003). Once the pollen grains have germinated, the pollen tubes grow down the style into an ovule where fertilisation of the egg cell (to form a zygote) and polar nuclei of the egg sac (to form the endosperm) occur (Dennis, 2003). Successful fertilisation depends on the pollen grains reaching the ovule before it degenerates.

The time during which the flower can be fertilised, if pollination is not limited, is assessed by the effective pollination period (EPP) (Williams, 1966). The EPP is defined as the number of days during which pollination is effective in producing a fruit and is determined under orchard conditions by recording the initial fruit set (fruit set after pollination) and final (mature) fruit set in hand-pollinated flowers. Variation in EPP values has been claimed to be due both to environmental effects, mainly temperature (Tromp and Borsboom, 1994) and to flower quality (Jackson, 2003). The EPP values are highly variable among cultivars, years and sites. Typically, EPP values vary between 2 and 9 days (Sanzol and Herrero, 2001).

2.2.1.3. *Gametophytic self-incompatibility, cross-incompatibility and semi-incompatibility*

Fruit set in most apple cultivars is less than 10% after self-pollination (Komori et al., 1999). Self-fertilisation is inhibited due to the presence of a multi-allelic *S* locus, which contains pistil *S* and pollen *S* genes, that is responsible for S-RNase-mediated gametophytic self-incompatibility (GSI) (see Sassa, 2016 and reference therein). Cultivars with the same *S* alleles cannot fertilise each other. When a *S* haplotype in the haploid pollen matches one of the two *S* haplotypes in the pistil, then the pollen is recognised as “self” and the pollen tube formation is blocked at the upper part of the style, whereas the “non-self” pollen tubes can grow along the style and reach the ovary. Because of this self-incompatibility, outcrossing is promoted, resulting in the majority of cultivars displaying high levels of allelic heterozygosity. However, self-incompatibility in Rosaceae is broken down by polyploidisation and has been demonstrated in tetraploids of apple (Adachi et al., 2009).

Although many species are believed to be strongly self-incompatible, variations between species in self-incompatibility strength have been observed. The increase in self-compatibility in species with a functional self-incompatibility mechanism, like apple, can be caused by a variety of environmental variables such as temperature and flower age (Ferrer et al., 2009). Such species are called partial or pseudo-self-compatible, partially self-compatible or pseudo self-fertile (Levin, 1996). Self-incompatibility in apple can also vary in strength depending on the apple cultivar. De Witte et al. (1996) found ‘Fuji’ and ‘Golden Delicious’ to give only 1% and 1.8% set, respectively, following self-pollination under conditions, where pollination of these cultivars with the ornamental apple ‘Baskatong’ gave 24% and 25% set, respectively. Much higher levels of self-fertility were found in ‘Idared’ (12.3%) and ‘Elstar’ (7%) although very few of the resulting fruits contained seeds (De Witte et al., 1996).

Instances of cross-incompatibility and semi-compatibility exist (Janick and Moore, 1975; Ramírez and Davenport, 2013). These are also controlled by the *S* locus that plays a role in self-incompatibility. Depending on their *S* loci, pairs of apple cultivars can be incompatible

when both loci are identical and semi-compatible when they carry one different and one similar *S*-locus. Pairs of apple cultivars are fully compatible when they differ in their *S* loci.

2.2.2. *Pollination, pollen dispersal and pollen viability*

Pollen dispersal and pollination depend on the pollinator (pollen vector), weather conditions, surrounding habitat and polliniser (pollen donor tree).

2.2.2.1. *Pollinator*

Insects, most notably honeybees, but also bumblebees, other wild bees and to a lesser extent some flies are the primary vectors for pollination in apple. Apple pollen is relatively heavy and not easily carried by the wind (Dennis, 2003; Jackson, 2003).

Apple growers typically rent honeybee hives during the bloom period, and it is recommended that they are placed at a density of four or five strong colonies per hectare in a mature orchard (Dennis, 2003). Although honeybees are the insects most used in orchards they are not as efficient pollinators as some solitary bees and bumble bees. A lot of honeybees take nectar from the flower without even touching the anthers (Delaplane and Mayer, 2000) and do not contribute to pollination. Moreover, Sapir et al. (2017) found that adding bumblebees to honeybees increased cross-pollination. Adding bumblebees had multiple effects on pollination: not only the number of pollinating insects increased but these were now also working in adverse weather conditions, and even the foraging behaviour of the honeybees was enhanced.

2.2.2.2. *Weather*

Rain during the flowering period may have a negative impact on the pollination of apples, by lowering the foraging activities of pollinators which reduces pollen transfer (Abrol, 2012). Another possibility is that rain inhibits the germination and growth of pollen on the stigma (AHDB, 2017). Similarly, wind can also have negative influences on apple pollination. Strong winds will make it harder for pollinators to transfer pollen from flower to flower (Jackson, 2003). At wind speeds of 15 km/h or more, honeybees do not fly and the limited amount of pollen that would have been transferred will rapidly desiccate (Tromp, Webster and Wertheim, 2005).

During flowering a minimum temperature of 10°C is needed for effective pollen germination. When the temperature increases to 20°C, pollen germination rate (Yoder et al., 2009; Abrol, 2012) and hence pollen tube growth (Jefferies and Brain, 1984) increases with higher chances of successful fertilisation. Spring frosts can cause severe damage especially for early flowering cultivars, and the damage is not always visible immediately after frost (Jackson, 2003; Tromp, Webster and Wertheim, 2005).

2.2.2.3. *Surrounding habitat*

Small habitats in the environment support wild pollinators with food and housing and encourage the diversity of wild bees (Sheffield, Ngo and Azzu, 2016). The surrounding habitat needs to be diverse in plant species and provide the bees with adequate nectar and pollen while also offering suitable nesting opportunities (Garibaldi et al., 2013; Sheffield et al., 2013).

2.2.2.4. *Polliniser*

The polliniser (i.e., pollen donor tree) used in the orchards is also an important factor for fertilisation. There are two main aspects in choosing the right polliniser: bloom overlap and compatibility with the acceptor tree.

Bloom overlap takes place when two apple cultivars flower synchronously, promoting effective pollen transfer. When pollen is deposited before the receptive period, the pollen should remain viable and fertile for a long enough period to allow successful pollination. Differences in pollen viability has been reported for different cultivars (Petrisor et al., 2012; Moshtagh et al., 2015). Pollen fertility of most apple cultivars is close to 100%, but is reduced in some cultivars by unknown factors or triploidy.

The second important aspect is compatibility. In case of cross-incompatibility, not all cross-pollination will result in fertilisation and seed formation. Semi-compatibility, however, is not a problem if there are enough pollinators, if blossom abundance and pollen supply is high and conditions for pollination are good.

2.2.2.5. *Pollen dispersal and dispersal studies*

Many apple species are self-incompatible and, hence, fruit production only occurs with pollen transfer between cultivars. Most orchards consist of a limited number of cultivars, which are arranged in monotypic blocks or rows. The economic costs due to fruit yield loss as a result of inadequate pollination highlights the need to more accurately predict patterns of pollen movement in orchards (Kron et al., 2001). Pollen dispersal in apples has received considerable attention. However, most studies are based on apparent rather than realised pollen dispersal (Free and Spencer-Booth, 1964; Wertheim, 1991), and only few studies have attempted to relate factors other than distance to pollination success. In apple orchards, the majority of honeybee foraging flights are between flowers on the same tree and secondarily between adjacent trees in the same row and to a lesser extent across rows (Free and Spencer-Booth, 1964; Free, 1966). However, in a study by Kron et al. (2001) in which they have examined pollen dispersal by molecular markers, they found the same amount of seeds sired by the polliniser along and across the row.

Early research on pollen dispersal was conducted using a pollen donor carrying a dominant gene for red leaf colour, such as ‘Baskatong’. Researchers monitored gene flow in apple orchards by observing the percentages of red leafed seedlings borne from trees at increasing distances from the ‘Baskatong’ polliniser. A study using this approach found that 69% and 91% of the fertilised seeds occurred within the first 10 m and 60 m of the pollen donor, respectively (Reim et al., 2006). Kron et al. (2001) used allozyme markers to determine parentage of seeds to track pollen flow in orchards where ‘Idared’ was planted as polliniser. Pollen dispersal generally declined with increasing distance, as 50% of total seeds sired by ‘Idared’ occur within the first four rows, or approximately 20 m (Kron et al., 2001). In a survey of a wild population of the crab apple *M. sylvestris*, Larsen and Kjær (2009) used microsatellite loci data in conjunction with spatial distances of the individual trees in the population to monitor pollen movement in a natural environment. These authors found that successful pollination occurred mostly between nearby trees, with a median distance of about 23 m. The data suggest that the majority of cross-pollination occurs between receptive flowers and proximate pollen sources. A significant factor affecting pollination intensity is distance from the pollen source. Other important factors include, weather, pollinator presence, cultivar compatibility and flowering synchrony (Kron et al., 2001). Maximum pollen dispersal distances in orchard settings were reported up to 40 m (Wertheim, 1991), 86 m (Kron et al., 2001), 104 m (Reim et al., 2006), 137 m (Tyson,

Wilson and Lane, 2011) and 150 m (Soejima, 2007). In-hive transfer of viable pollen between bees foraging in geographically distant areas may explain long-distance pollen flow (Degrandi-Hoffman, Hoopingarner and Klomparens, 1986).

To predict bee-vectored pollen transfer, Tyson, Wilson and Lane (2011) developed a mechanistic model based on monitoring beta-glucuronidase (GUS) activity in seeds borne from trees located at increasing distances from a row of transgenic GUS-expressing 'Gala' trees. The authors use the model to examine the effect of buffer rows and isolation distances on outcrossing rates. The model demonstrates that the level of outcrossing is affected by the relative sizes of the nearby orchards. As the size of the orchard with conventional trees becomes smaller relative to the orchard with transgenic trees, the isolation distance required to limit the frequency of outcrossing is increased. Furthermore, incorporation of buffer rows between the two orchard types generally reduces the isolation distance required in order to limit outcrossing frequency (Tyson, Wilson and Lane, 2011).

2.2.3. Seed production and natural dispersal of fruits and seeds

2.2.3.1. Seed production

Seed production is the result of fertilisation of the egg cell present in the apple flower and the male gamete present in the pollen. Seeds develop and reside in the central core of the fruit, in the carpels. Each carpel has two ovules that develop into seeds following fertilisation. In general, the seed number of fruit varies between 2-7, depending on the fertilisation intensity.

2.2.3.2. Natural dispersal of fruits and seeds

The primary means of movement and dispersal of apple seeds in natural settings is through frugivorous mammals, such as bears, foxes and deer (Willson, 1993; Myers et al., 2004), as well as birds (Witmer, 1996 and references therein). For example, white-tailed deer travel a range of many hectares on a daily basis and are considered dispersers of low numbers of apple seeds (Myers et al., 2004). Seeds may accidentally be attached to vertebrates or fruits could be ingested and digested, thereby deliberating the seeds (Galetti, 2002; Herrera, 2002; Myers et al., 2004). Seeds pass through the digestive tract of animals, and can germinate and generate a new apple tree. It is also possible that apple seeds are dispersed by humans during the transport fruit or after eating the fruit.

2.2.4. Seed viability, germination, seedling viability and establishment

In apple it is accepted that there is no seed bank formed in nature, although this has not been studied in detail. During the winter, cold temperatures (< 5°C) and high humidity break the dormancy of mature seed. This process is known as stratification. The stratification time depends on the genotype. Seeds will either germinate during spring or die. Seed germination rate is very high and can reach up to 95% under controlled conditions of stratification and sowing.

The survival of seedlings in nature is rather low, but precise data are not available. Seedling growth in the first year is minimal compared to the later years. Seedlings can grow in very different soil and climate conditions of temperate regions with enough precipitation, although they can grow also in semi-arid regions.

2.2.5. Asexual propagation

2.2.5.1. Apomixis

Apomixis, a form of asexual reproduction in flowering plants, is common in agriculturally important crops, except for apple and Citrus. Apomixis *sensu stricto* refers to asexual seeds that are genetically identical to the mother plant. Apples are facultative apomicts which means that they contain both sexually and asexually produced seeds in their fruits. The process of apomixis is very similar to sexual reproduction and also leads to viable seeds. However, in contrast to sexual reproduction, there is no fusion of male and female gametes and embryo sac formation occurs without meiosis. The embryo is thus only derived from maternal somatic tissue and is therefore a clone of the mother plant (Koltunow, 1993).

2.2.5.2. Vegetative reproduction

Another form of asexual reproduction in apple trees is the formation of root suckers. Root suckers are sprouts that arise from the roots. Because commercial apple trees are planted on rootstocks, these root suckers are originating from the rootstock and not from the commercial cultivar itself. Several commercial rootstocks, including M.7, M.9 and G.202, tend to produce a lot of root suckers which need to be removed regularly because they compete for water and nutrients with the commercial tree and because they form a potential entry point for fire blight (Miller and Racsko, 2011). These root suckers will not have the chance to flower in commercial orchards. However, after felling, the apple tree's remaining roots can produce root suckers that flower after several years when the land is not further managed.

2.2.5.3. Parthenocarpy

Apples can develop fruits without fertilisation, known as parthenocarpic fruits. Parthenocarpic fruits are seedless. There are two distinct types of parthenocarpy: vegetative and stimulative parthenocarpy. Vegetative parthenocarpy is spontaneous and arises without pollination or any other externally applied stimulus. Several apple cultivars are intrinsically parthenocarpic, e.g., 'Spencer Seedless', but they are rarely of economic value (Jackson, 2003). Stimulative parthenocarpy can be induced by pollination without fertilisation (e.g., with irradiated pollen), or by plant hormones, primarily gibberellins. Although parthenocarpy can be induced with growth regulators in apple, the response is limited and such treatments are not used commercially (Jackson, 2003).

SECTION 3. GENETICS

3.1. Detailed genetic information

Whereas the haploid (x) chromosome numbers of most species in the family Rosaceae are 7, 8 or 9, the Maleae tribe, including *Malus domestica*, is distinct in having a haploid chromosome number of 17 ($x = 17$). Two hypotheses are still under investigation to explain the chromosome number of Maleae. The first hypothesis postulates that Maleae originated from ancient hybridisations between species in the Prunoideae ($x = 8$) and the Spiraeoideae ($x = 9$), followed by chromosome doubling. The original hybrids would have been sterile and only after chromosome doubling would they have formed fertile allopolyploids (Way et al., 1990; Luby, 2003; Webster, 2005a). The second hypothesis postulates that the genome originated >50 million years ago from a genome-wide duplication event of the $x = 9$ ancestral (most probably) *Gillenia* chromosomes followed by loss of one chromosome (Velasco et al., 2010). As a genome-wide duplication pattern was found for all chromosomes (Velasco et al., 2010; Daccord et al., 2017), this hypothesis likely explains the 17 chromosomes in *M. domestica*.

Most *Malus* species, including *M. domestica*, are diploid with $2n = 34$ chromosomes, however, different levels of ploidy (tri-, tetra-, penta- and hexaploidy) are also known (Höfer and Meister, 2010). The mean value for the 2C nuclear DNA content of *M. domestica* is 1.514 pg per nucleus (Höfer and Meister, 2010).

The nuclear genome of *M. domestica* cultivar ‘Golden Delicious’ has been sequenced and represents the apple reference genome (Velasco et al., 2010; Daccord et al., 2017). Using the ‘Golden Delicious’ reference, nearly 200 apple genotypes have been re-sequenced. In addition to the nuclear DNA, whole organelle genomes (chloroplast and mitochondrial DNA) of apple were also sequenced (Goremykin et al., 2012; Nikiforova et al., 2013; Duan et al., 2017).

Genetic linkage mapping has contributed significantly to our current understanding about the structure of the *Malus* genome. First genetic linkage studies on apple were done in the late 1980s (Chevreau and Laurens, 1987; Manganaris and Alston, 1987; 1988a,b) and were followed by international apple genome mapping projects in Europe (King et al., 1991) and New Zealand (Gardiner et al., 1996). The first linkage map of the apple genome was published by Hemmat et al. (1994). With the establishment of simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, the number of linkage studies increased rapidly. Today, there are more than 60 different apple linkage maps available in the Genome Database for Rosaceae (<https://www.rosaceae.org/>). These maps were established using different parental genotypes, with different types of DNA markers, and were established for traits ranging from disease resistance to fruit quality, aroma, flower and fruit development, harvesting time and tree growth parameters, etc. The biggest advance in this field of research is the development of a multi-parental, high-density, integrated Genetic Linkage Map (iGLMap) of apple, comprising 15 417 SNP markers (Di Pierro et al., 2016).

The first apple reference transcriptome was published by Velasco et al. (2010) and significant improvements were made by Bai, Dougherty and Xu (2014). The use of high-throughput proteomics and metabolomics approaches in apple research has been increasing exponentially during the last few years (for reviews see Liu et al. (2017)).

3.2. Breeding approaches

The commercial apple tree, as in the case for other fruit trees, is a composite of a rootstock and a scion. The rootstock constitutes the root system and a small proportion of the lower trunk whereas the scion grafted or budded onto the rootstock forms the upper fruiting part (Webster and Wertheim, 2003). Rootstocks influence the performance of the grafted scion and can affect traits like drought and disease resistance of the tree, and vigour, precocity, and fruiting of the scion (Webster and Wertheim, 2003). Various compounds, such as minerals, proteins, ribonucleoproteins, RNA and small RNAs are transferred between the rootstock and scion, but the molecular mechanisms of how the rootstock influences scion phenotypes is not yet clarified in detail (Kumari et al., 2015).

The scion is the cultivar or the part of the tree that has name recognition, such as ‘Jonagold’. Once a commercial cultivar is obtained, they are always propagated asexually (Webster and Wertheim, 2003). Sexual propagation is not preferred as each seed is genetically unique with considerably different properties of the parental genotypes, which would result in a plant that is not ‘true-to-type’. Additionally, cultivars propagated from seeds generally bear fruits of poor size, appearance and quality. Although rootstocks can be propagated via seeds, the propagation of rootstocks is becoming increasingly asexual (Tromp, Webster and Wertheim, 2005). Rootstocks and scions are generally *Malus* species or interspecific *Malus* hybrids.

Breeding in apple is focused on the development of better rootstock and scion cultivars. The main accomplishment in apple rootstock breeding was the development of dwarfing apple rootstocks, which started in the early 1920s, by the East Malling Research Station in Kent, England (Tukey, 1964; Mudge et al., 2009). The M.9 rootstock, released by this Research Station, and its improved selections belong until now to the most used rootstocks worldwide. Other efforts in rootstock breeding resulted, for example, in the selection of dwarfing rootstocks tolerant to winter cold or in resistance to fire blight, collar rot and other diseases (Cummins and Aldwinckle, 1994). In apple scion breeding, obtaining a combination of high fruit quality (fruit size, inner fruit quality, fruit colour, flavour and aroma) with disease and pest resistance is the main objective. Furthermore, a range of other traits is considered important in the selection of new (scion) cultivars, like high and regular yielding, the percentage of marketable fruits (Peil et al., 2011), adaptation to climatic conditions, and storability of fruits. Low allergenic or high flavonoid content, red fruit flesh or low chilling are minor objectives.

There are several characteristics of *M. domestica* that inhibit rapid genetic improvement of cultivars, most notably: a long juvenile period, self-incompatibility, the high level of heterozygosity resulting from the necessary cross-pollination and inbreeding depression (Brown and Maloney, 2003). The breeding programs follow, in general, a common strategy, which establishes a series of back-crossings between hybrids exhibiting a trait of interest and susceptible commercial cultivars. Usually, the source of resistance genes comes from sexually compatible wild apple relatives. The traditional breeding method that is most commonly used is a modified backcross, where a different recurrent parent is used in each generation of backcrossing to circumvent self-incompatibility. This process is laborious and time consuming because it demands several generations to recover near-isogenic lines of high commercial value expressing the desired trait. Typically, the process takes more than 20 years. For example, it took several decades to successfully introgress a scab resistance trait from crab apple into a commercial cultivar (Gessler and Pertot, 2012).

Recent advances in genomic technologies allow for acceleration of the development of cultivars of apple. Modern breeding programs using molecular tools have shown to allow for early screening (typically at the seedling stage) for certain inherited traits, like scab resistance (e.g., Bus et al., 2000; Patocchi et al., 2009; Baumgartner et al., 2015) without the need to phenotype the plants themselves. Two important international research programmes, ROSBREED (www.rosbreed.org) in the United States and FruitBreedomics (www.fruitbreedomics.com) (Laurens et al., 2012) in Europe, have provided such molecular tools for screening and developed pre-breeding material. Although simple sequence repeats (SSR) are still used for marker-assisted selection of traits, the development of single nucleotide polymorphism (SNP) markers (Jansch et al., 2015) and the establishment of an apple 480k SNP genotyping array (Bianco et al., 2016) can allow for genomic selection of traits, such as fruit quality traits as demonstrated by Kumar et al. (2012).

Recombinant DNA technology developments can also address some of the breeding bottlenecks in the development of elite cultivars and rootstocks of apple (for more information see Annex B). Successful reduction of the juvenile period to one single year was achieved via constitutive expression of the *BpMADS4* gene from silver birch in early flowering apple lines (Flachowsky et al., 2007, 2009, 2011, 2012). Further, it is a promising tool as it can be used for the direct introduction of specific genes (traits) into a particular cultivar while retaining all other desirable qualities. For example, scab-resistant transgenic apple cultivars were obtained via *Agrobacterium*-mediated transfer and further attempts resulted in the first successful report of a cisgenic apple (Vanblaere et al., 2011).

SECTION 4. HYBRIDISATION AND INTROGRESSION

4.1. Natural facility of interspecific crossing (extent, sterility and fertility)

Most species in the genus *Malus* can be readily hybridised after artificial cross-pollination (Luby, 2003; Hancock et al., 2008), but interspecific crossing can also occur under natural conditions. The latter is the case in forests in Flanders where 7% of the sampled *Malus sylvestris* trees were hybrid forms of *M. sylvestris* and *M. domestica* (Keulemans, Roldan-Ruiz and Lateur, 2007). The capacity for inter-species hybridisation within the genus *Malus* is evident by the numerous hybrids among *Malus* spp. (e.g., Korban, 1986; Schuster and Büttner, 1995; USDA-ARS, 2018). The majority of *Malus* spp. are diploid and inter-fertile, as there are no apparent physiological or genetic barriers (Korban, 1986; Vanwynsberghe, 2006). Triploid and tetraploid commercial apple cultivars exist, but introgression of their genes into wild species seems unlikely since triploids are considered sterile and tetraploid introgression in diploid wild species will give sterile triploid progeny.

Outside of the genus *Malus*, the potential for natural hybridisation with other genera appears to be limited. While there has been extensive intergeneric hybridisation reported among closely related taxa (e.g., in the former subfamily Maloideae), a summary presented by Robertson et al. (1991) indicates no intergeneric crosses involving *Malus* spp. outside of breeding programs. A reported *Malus* × *Chaenomeles* hybrid was subsequently discounted by Rudenko (1976, cited in Robertson et al., 1991), and a proposal that the species *M. florentina* (Zuccagni) C. K. Schneid. was the product of hybridisation between *Malus* and *Sorbus* sect. *Torminaria* (called × *Malosorbus*) was also subsequently challenged by several authors who considered it a relictual species of *Malus* (e.g., Huckins, 1972, cited in Robertson et al., 1991). This has been further supported by more recent taxonomic work (Qian et al., 2008).

4.2. Experimental crosses

Artificial interspecific hybrids are easily produced (Luby, 2003). *M. domestica*, which is thought to be of hybrid origin (Korban, 1986), can be readily hybridised with its congeners in the genus *Malus* (Korban, 1986; Vanwynsberghe, 2006; Kron and Husband, 2009). Interest in controlled hybridisation for the improvement of cultivated apples dates back to the 1700s, and reports of successful experimental interspecific hybridisations began in the late 1800s (Korban, 1986). Since then, interspecific hybridisation has played a major role in genetic improvement and a large number of crosses have been made among *Malus* spp. in research and breeding programs throughout the world, primarily to improve the cultivated apple or to develop new hybrid species with distinctive characteristics (Korban, 1986). A list of experimental interspecific hybrids that have been documented in the genus *Malus* is provided by Korban (1986), and includes about 60 different species combinations. Some new interspecific combinations with *M. domestica* have been added to this list (Vanwynsberghe, 2006).

Breeding programs have produced intergeneric hybrids between apple and pear (*Malus* × *Pyrus*), and apple and hawthorn (*Malus* × *Crataegus*) as reported in Robertson et al. (1991), however, these relied on techniques such as embryo rescue (Banno et al., 2003). Forced intergeneric hybridisation of *Cydonia* (quince) with *Malus* resulted in fertile genotypes which have been identified as an artificial hybrid genus × *Cydomalus*. However, the

seedlings produced are generally weak and of low viability and germinability (Bell and Leitão, 2011). F2 progeny from F1 genus × *Cydomalus* hybrids seem to originate from apomictic seeds (Keulemans, personal communication). Hybridisation barriers between *Malus* and related species are complex and involve several processes: reduced pollen germination, although pollen adhesion on the stigma seems not affected, reduced pollen tube growth in the style and the ovary, and incongruity of pollen and egg cell. Almost no seeds are formed after an intergeneric cross and seeds are in most cases not viable (Vanwynsberghe, 2006).

4.3. Information and data on introgression

A number of studies show that there is a potential for gene introgression from *M. domestica* into native *Malus* species. Coart et al. (2003, 2006) evaluated hybridisation between *M. domestica* and the European wild crab apple *M. sylvestris* in Belgium using nuclear microsatellites and found that 11% of the sampled *M. sylvestris* trees were of hybrid origin. Larsen et al. (2006) on the other hand, did not find *M. domestica* × *M. sylvestris* hybrid individuals in natural *M. sylvestris* populations in Denmark despite the overlap in geographical distribution and flowering time of *M. domestica* and *M. sylvestris*. The fact that handmade interspecific crosses between these two species yield viable seeds which exhibited normal growth and development up to young seedlings suggests there is some other, still unknown, reproductive barrier operating to maintain genetically distinct populations (Larsen, Jensen and Kjær, 2008). A study conducted by Kron and Husband (2009) in southern Ontario examined populations of the introduced *M. domestica* and the native tetraploid crab apple *M. coronaria* and found that their geographic ranges and flowering times overlapped sufficiently for cross-pollination to occur. The study found that 27.7% of seeds from open-pollinated fruit was of hybrid origin. However, the ability of the resulting hybrid plants to survive and backcross with *M. coronaria* is unknown at this time, and the adult trees within the populations were all identified to be distinct species (either *M. domestica* or *M. coronaria*). Successful backcrosses seem unlikely since the hybrids are expected to be triploids, which are normally not fertile.

SECTION 5. GENERAL INTERACTIONS WITH OTHER ORGANISMS AND ECOLOGY

5.1. Interaction with natural and other ecosystems where the species is cultivated or managed

Interactions between cultivated apple *Malus domestica* and other organisms include those with agricultural diseases and pests, beneficial organisms, soil organisms, as well as with other *Malus* species. For the latter we refer to 4.1.

5.1.1. Pests and diseases

M. domestica is susceptible to a number of fungal and bacterial plant diseases (see Annex C). The most economically important disease of apples worldwide, is apple scab caused by the fungal pathogen *Venturia inaequalis* (Cooke) Wint. Another significant disease reported in all major apple production regions with a widespread yearly occurrence and high pest pressure is fire blight caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al. Fire blight can, under the right conditions, wipe out entire orchards within a growing season (AAFC, 2013). Other significant fungal diseases reported as having localised yearly occurrence with high pest pressure or widespread sporadic occurrence with high pest pressure include, respectively, black rot (in Ontario), caused by the *Botryosphaeria obtusa* (Schwein.) Shoemaker; and powdery mildew, caused by the *Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon (AAFC, 2013). Viruses and viroids can occur in apple orchards, but many of them are symptomless in most commercial cultivars. The apple mosaic virus (APMV) is one of the most widespread apple viruses (Reddy, 2010).

A variety of pests can threaten apple trees and fruits (Carlson, 2008; Sherwani, Mukhtar and Wani, 2016). One of the most common pests of apples, is the codling moth (*Cydia pomonella*). This insect eats holes and burrows the core of the apple fruit. Different scale insects, including the San Jose scale (*Quadraspidiotus perniciosus*) and many bugs, like the brown marmorated stink bug (*Halyomorpha halys*), living on apple, impact fruit quality. A group of insects that damages fruit trees are mites. The European red mite (*Panonychus ulmi*), the two-spotted spider mite (*Panonychus urticae*) and the apple rust mite (*Aculus schlechtendali*) are major mite pests in apple orchards. Leaf rollers can damage both fruits and leaves, while the woolly apple aphid (*Eriosoma lanigerum*) affects leaves, bark and rootstocks. An overview of pests is presented in Annex D.

Some vertebrates can also be considered pests to apple growers. Birds, such as pigeons and crows, can peck holes in the fruit or wood (Tromp, Webster and Wertheim, 2005; AAFC, 2013).

5.1.2. Beneficial insects

5.1.2.1. Pollinators

The interaction between honey bees and bumble bees and apple trees are mutual beneficial: the bees aid in fertilisation (see 2.2.2.1) and receive nectar and pollen in return.

5.1.2.2 Biological control organisms

In managed systems one can exploit the mutual interactions between organisms living on apple and the apple host. In some specific cases, it is possible to manage and control pests and diseases with biological control organisms (BCOs). The application of BCOs in cultivated apple has been investigated in several parts of the growth cycle, from flowering (Pusey, Stockwell and Mazzola, 2009) to post-harvest storage (Jamalizadeh et al., 2011). The use of arthropods against pests which damage the leaves and fruits of the apple plants, such as aphids and mites, is common (Asante, 1997; Nicholas, Spooner-Hart and Vickers, 2005; Brown and Mathews, 2007; Zhou et al., 2014; Walker, Suckling and Wearing, 2017). Even birds can be deployed as BCOs for the predation of caterpillars (Mols and Visser, 2002), but this measure is rarely applied in commercial orchards. This is also the case for bacteria that have been used as BCOs to suppress pathogens like blue mould (Etebarian et al., 2005) and grey mould (Jamalizadeh et al., 2008), or for the fungus *Trichoderma* to control *Phytophthora* in apple seedlings (Roiger and Jeffers, 1991).

Alternative strategies are under investigation to use BCOs as antagonists against *E. amylovora*. One approach for instance is the use of bumblebees as vector to bring the BCOs to the flowers during bloom (entomovectoring) (Remy et al., 2016, 2017).

5.1.3. Animals

Various mammals, including rodents, rabbits, hares and deer feed on tree tissues, including girdling of bark and feeding on roots, young branches, leaves and buds (AAFC, 2013). Various birds, such as crows and woodpeckers, and some mammals, including bears, feed on fruits.

Apple seeds contain small amounts of amygdalin, a cyanogenic glycoside, which is a naturally occurring plant pre-toxin considered to play a role in plant defence against herbivores due to bitter taste and release of toxic hydrogen cyanide upon tissue disruption (Dar et al., 2016). The presence of small amounts of toxicants and other metabolites (organic acids, phenolic compounds) in apple and products derived from apple, and the effect this may have on humans are addressed in the OECD Consensus Document on Compositional Considerations for New Cultivars of Apple (OECD, 2019).

5.1.4. Soil micro-organisms

Soil micro-organisms interacting with apple are poorly studied. In most cases rhizosphere micro-organisms are studied in relation to replant diseases in commercial apple plantations (e.g., Čatská et al., 1982; Jiang et al., 2017) or specific treatments on the crop like triazole fungicides (Sulowicz and Piotrowska-Seget, 2016). In some cases, rhizosphere micro-organisms can be used for biological control of soil borne diseases in apple (Sindhu, Rakshiya and Sahu, 2009) or to improve growth and nutrient uptake (Rengel and Marschner, 2005; Rumberger, Merwin and Thies, 2007).

Annex A. *Malus* Species

Table A.1. Species and hybrid species in the genus *Malus*

Latin name	Common name (English)
1 <i>M. × adstringens</i> Zabel	
2 <i>M. angustifolia</i> (Aiton) Michx	Southern crab apple
3 <i>M. × arnoldiana</i> (Rehder) Sarg. ex Rehder	
4 <i>M. × asiatica</i> Nakai	
5 <i>M. × astracanica</i> (hort. ex Dum) Cours	
6 <i>M. × atrosanguinea</i> (hort. ex Späth) C. K. Schneid.	
7 <i>M. baccata</i> (L.) Borkh.	Siberian crab apple
8 <i>M. baoshanensis</i> G. T. Deng	
9 <i>M. brevipes</i> (Rehder) Rehder	
10 <i>M. chitralensis</i> Vassilcz.	
11 <i>M. coronaria</i> (L.) Mill.	Sweet crab apple
12 <i>M. crescimannoi</i> Raimondo	
13 <i>M. × dawsoniana</i> Rehder	
14 <i>M. domestica</i> Borkh.	Apple
15 <i>M. doumeri</i> (Bois) A. Chev.	
16 <i>M. florentina</i> (Zuccagni) C. K. Schneid.	Hawthorn-leaf crab apple
17 <i>M. floribunda</i> Sieb. ex Van Houtte	Japanese crab apple
18 <i>M. fusca</i> (Raf.) C. K. Schneid.	Oregon crab apple
19 <i>M. × gloriosa</i> Lemoine	
20 <i>M. halliana</i> Koehne	Hall crab apple
21 <i>M. × hartwigii</i> Koehne	
22 <i>M. honanensis</i> Rehder	
23 <i>M. hupehensis</i> (Pamp.) Rehder	Chinese crab apple, Hupeh crab
24 <i>M. ioensis</i> (Alph. Wood) Britton	Iowa crab apple, prairie crab apple
25 <i>M. kansuensis</i> (Batalin) C. K. Schneid.	
26 <i>M. komarovii</i> (Sarg.) Rehder	
27 <i>M. leiocalyca</i> S. Z. Huang	
28 <i>M. × magdeburgensis</i> Hartwig	
29 <i>M. mandshurica</i> (Maxim.) Kom. ex Skvortsov	Manchurian crab apple
30 <i>M. × micromalus</i> Makino	Kaido crab apple
31 <i>M. × moerlandsii</i> Door.	
32 <i>M. muliensis</i> T. C. Ku	
33 <i>M. ombrophila</i> Hand.-Mazz.	
34 <i>M. orientalis</i> Uglitzk.	
35 <i>M. orthocarpa</i> Lavalley ex anon.	
36 <i>M. × platycarpa</i> Rehder	
37 <i>M. prattii</i> (Hemsl.) C. K. Schneid.	
38 <i>M. prunifolia</i> (Willd.) Borkh.	Chinese crab apple, plum-leaf crab apple
39 <i>M. pumila</i> Mill.	Paradise apple
40 <i>M. × purpurea</i> (A. Barbier) Rehder	
41 <i>M. × robusta</i> (Carrière) Rehder	Siberian crab apple
42 <i>M. sargentii</i> Rehder	Sargent's crab apple
43 <i>M. scheiderckeri</i> (L. H. Bailey) Späth ex Zabel	
44 <i>M. sieversii</i> (Ledeb.) M. Roem.	

45	<i>M. sikkimensis</i> (Wenz.) Koehne ex C. K. Schneid.	
46	<i>M. × soulardii</i> (L. H. Bailey) Britton	Soulard crab apple
47	<i>M. spectabilis</i> (Aiton) Borkh.	Asiatic apple, Chinese crab apple
48	<i>M. spontanea</i> (Makino) Makino	
49	<i>M. × sublobata</i> (Dippel) Rehder	
50	<i>M. sylvestris</i> (L.) Mill.	European crab apple
51	<i>M. toringo</i> (Siebold) de Vriese	Toringo crab apple
52	<i>M. toringoides</i> (Rehder) Hughes	
53	<i>M. transitoria</i> (Batalin) C. K. Schneid.	
54	<i>M. trilobata</i> (Poir.) C. K. Schneid	
55	<i>M. tschonoskii</i> (Maxim.) C. K. Schneid.	
56	<i>M. × xiaojinensis</i> M. H. Cheng & N. G. Jiang	
57	<i>M. yunnanensis</i> (Franch.) C. K. Schneid.	Yunnan crab apple
58	<i>M. zhaojiaoensis</i> N. G. Jiang	
59	<i>M. zumi</i> (Matsum.) Rehder	

Source: USDA-ARS (2018).

Annex B. Biotechnological Developments

Apple has become a model species for Rosaceae genetic and genomic research. James et al. published in 1989 the first *Agrobacterium tumefaciens*-mediated leaf disc transformation in apple. In the subsequent years, the main objective in several laboratories around the world was to improve the methodology and to create a “clean vector technology” for marker-free transgenic apples. Due to the lack of availability of “apple own” genes giving a commercially interesting advantage (e.g., disease resistance), the first achievements in genetic engineering of apple (from the early 1990s on) mainly relied on genes from other species and have been reviewed by Gessler and Patocchi (2007), Hanke and Flachowsky (2010) and Rai and Shekhawat (2014). The main target traits in apple transformation are disease and pest resistance, in particular fungal resistance to scab and powdery mildew and resistance to the bacterial fire blight disease. Other traits in apple on which research has been conducted include: (a) stress tolerance, (b) herbicide resistance, (c) self-incompatibility, (d) fruit ripening and other fruit characteristics, (e) allergens, (f) precocity and flower induction, and (g) dwarfing and rooting ability in rootstock genotypes.

To date, only few apple genes of potential interest have been mapped. Four disease resistance genes of apple have become available for transformation: two genes (*Rvi6*, Belfanti et al., 2004; *Rvi15*, Schouten et al., 2014) providing resistance to apple scab, one-gene (*Fb-Mr5*, Broggin et al., 2014) leading to fire blight resistance and one gene (*Pl2*, Rikkerink et al., 2016) mediating resistance to powdery mildew. Other candidate resistance genes have been identified, but map-based cloning of these genes is still in progress (Gessler and Pertot, 2012; Broggin et al., 2014; Schouten et al., 2014). Although most efforts have been done on the cloning of *R*-genes, a few genes encoding for other traits in apple have also been identified, like the *Ma* genes controlling the content of malic acid in apple fruits and the *Co* gene leading to a columnar like growth habit of the tree (Xu et al., 2012; Okada et al., 2016). Initiatives, such as TranscrApple (<http://www.transcrapple.com/en/en/home/>), may increase the amount of genes that become available for transformation.

Transgenic apples on the market and transgenic apples for which biosafety research is ongoing are summarised below:

Disease resistance: Scab-resistant transgenic apple cultivars were obtained via *Agrobacterium*-mediated transfer of scab resistance genes *Rvi6* from *M. floribunda*, a species of crab apple that shows natural resistance to some strains of the apple scab fungus, to cultivar ‘Gala’ (Barbieri et al., 2003; Belfanti et al., 2004; Silfverberg-Dilworth et al., 2005; Malnoy et al., 2008). Further research resulted in the first intragenic and cisgenic apple lines of the cultivar ‘Gala’ with scab resistance (Joshi et al., 2011; Vanblaere et al., 2011, 2014; Krens et al., 2015), containing the *Rvi6* gene from *M. floribunda* under the control of the promoter from the apple Rubisco gene or the native *Rvi6*-promoter, respectively. These plants were planted in a field trial to study scab resistance in an orchard situation (Krens et al., 2015). In 2015, the development of the first cisgenic apple with increased resistance to fire blight was reported (Kost et al., 2015). A cisgenic apple line C44.4.146 of the susceptible apple cultivar ‘Gala Galaxy’ was regenerated using the *FB_MR5* gene from the wild apple *M. robusta*.

Breeding cycle acceleration: Fruit trees typically have long breeding cycles. Successful reduction of the juvenile period of apple to one single year was achieved via constitutive expression of the *MADS4* transcription factor gene from silver birch (*Betula pendula* Roth.) in early flowering apple cultivars ‘Pinova’ (Flachowsky et al., 2007, 2009, 2011, 2012), ‘Gala’, ‘Mitchgl Gala’ and ‘Santana’ (Weigl et al., 2015). The BpMADS4-based breeding technology allows for more rapid introgression of agronomically relevant traits (e.g., disease resistances) from wild apples into domestic apple cultivars (e.g., Schlathölter et al., 2018).

Fruit quality: Apples have been engineered using a gene silencing technique called ribonucleic acid interference (RNAi), in order to reduce the production of polyphenol oxidases (PPO), which causes the apple’s flesh to brown when sliced or bitten (Xu, 2013). Transgenic versions of the varieties ‘Golden Delicious’, ‘Granny Smith’, and ‘Fuji’ have been approved for marketing (OECD, 2019).

Annex C. Apple Diseases

The following lists include the most relevant diseases in terms of economic losses.

Bacteria (including phytoplasma)

Latin name	Common name (English)
<i>Agrobacterium rhizogenes</i>	Hairy root
<i>Agrobacterium tumefaciens</i>	Crown gall
<i>Erwinia amylovora</i>	Fire blight
Phytoplasma	Apple chat fruit
<i>Pseudomonas syringae</i>	Blister spot
Apple-proliferation phytoplasma	

Fungi

Latin name	Common name (English)	Occurrence
<i>Alternaria alternata</i>	Alternaria rot	W
<i>Alternaria mali</i>	Alternaria blotch	A, AF, NA
<i>Armillaria mellea</i>	Root rot	W
<i>Athelia rolfsii</i>	Southern blight	AF, NA, SA
<i>Biscogniauxia marginata</i>	Blister canker	NA
<i>Botryosphaeria berengeriana</i>	Apple ring rot and canker	A
<i>Botryosphaeria dothidea</i>	Apple ring rot	AF, NA, SA, O
<i>Botryosphaeria stevensii</i>	Black rot, frog eye leaf spot, canker	AF, E, NA, SA, O
<i>Botrytis cinerea</i>	Grey-mould rot	W
<i>Butlerella eustacei</i>	Fish-eye rot	A, E, NA
<i>Cadophora malorum</i>	Side rot	A, NA
<i>Cladosporium</i> spp.	Mouldy core, core rot	W
<i>Colletotrichum</i> spp.	Bitter rot	E
<i>Corticium stevensii</i>	Thread blight	NA
<i>Cytospora ceratosperma</i>		A
<i>Diplocarpon mali</i>	Marssonina blotch	A, E, NA
<i>Epicoccum</i> spp.	Mouldy core, core rot	W
<i>Fusarium</i> spp.		E
<i>Geastrumia polystigmatis</i>	Sooty-blotch complex	W
<i>Gloeopeniophorella sacrata</i>	Peniophora root canker	O
<i>Grovesinia moricola</i>		A
<i>Gymnosporangium clavipes</i>	Quince rust	NA
<i>Gymnosporangium globosum</i>	American hawthorn rust	NA
<i>Gymnosporangium juniperi-virginianae</i>	Cedar apple rust	NA

<i>Gymnosporangium libocedri</i>	Pacific coast pear rust	NA
<i>Gymnosporangium yamadae</i>	Japanese apple rust	A
<i>Helicobasidium longisporum</i>		O
<i>Helminthosporium papulosum</i>	Black pox	NA
<i>Lepteutypa cupressi</i>	Monochaetia twig canker	NA
<i>Leptodontidium trabinellum</i>	Sooty-blotch complex	
<i>Leucostoma cinctum</i>	Leucostoma canker, dieback	E, NA
<i>Monilinia fructigena</i>	European brown rot	A, AF, E
<i>Monilinia laxa</i>	European brown rot	A, AF, E
<i>Monilinia mali</i>	Monilinia leaf blight	A
<i>Mucor</i> spp.	Mucor rot	
<i>Mycosphaerella pomi</i>	Brooks fruit spot	NA
<i>Nectria cinnabarina</i>	Nectria twig blight	E, NA, O
<i>Nectria ditissima</i>	Nectria canker	A, AF, NA, SA, O
<i>Neofabraea malicorticis</i>	Anthracnose canker, bull's eye rot	E, NA, O
<i>Paraconiothyrium fuckelii</i>	Blossom-end rot, Leptosphaeria canker, fruit rot	W
<i>Peltaster fruticola</i>	Sooty-blotch complex	W
<i>Penicillium</i> spp.	Blue mold	
<i>Peyronella obtuse</i>	Black rot, frog eye leaf spot, canker	AF, E, NA, SA, O
<i>Phacidiopycnis malorum</i>		E
<i>Phomopsis prunorum</i>	Phomopsis canker, fruit decay, rough bark	A, E, NA
<i>Phyllosticta solitaria</i> (Blotch)		NA
<i>Podosphaera leucotricha</i>	Powdery mildew	W
<i>Rosellinia necatrix</i>	Rosellinia root rot	W
<i>Schizothyrium pomi</i>	Fly-speck	W
<i>Scytinostroma galactinum</i>	White rot	NA
<i>Sphaeropsis</i> spp.		E
<i>Stemphilem</i> spp.		E
<i>Trichothecium roseum</i>	Pink mould rot	W
<i>Venturia inaequalis</i>	Apple scab	W
<i>Xylaria mali</i>	Black root rot	NA
<i>Xylaria polymorpha</i>	Black root rot	W

Note: A: Asia, AF: Africa, E: Europe, NA: North America, O: Oceania, SA: South America, W: worldwide.

Protista; Oomycota

Latin name	Common name (English)
<i>Phytophthora cactorum</i>	Phytophthora crown, collar, root and fruit rot
<i>Phytophthora syringae</i>	Phytophthora crown, collar, root and fruit rot

Viruses & viroids

Common name (English)	Acronym
Apple chlorotic leaf-spot virus	ACLSV
Apple stem-grooving virus	ASGV
Apple mosaic virus	APMV
Tulare apple-mosaic ilarvirus	TAMV
Apple stem-pitting foveavirus	ASPV
Tomato ringspot nepovirus	TomRSV
Apple fruit-crinkle viroid	AFCVd
Apple dimple-fruit viroid	ADFVd
Apple scar-skin viroid	ASSVd

Sources: Ogawa and English (1991), Grove et al. (2003), Hadidi et al. (2003), Betere Bomen (2018), VIDE (2018), Groenkennisnet (2019).

Annex D. Apple Pests

Arthropoda (ranked by order)

Latin name	Common name (English)
Coleoptera (beetles, weevils)	
<i>Anthonomus piri</i>	Apple bud weevil
<i>Anthonomus pomorum</i>	Apple blossom weevil
<i>Curculionidae nenuphar</i>	
<i>Polydrusus</i>	
<i>Sitona lineatus</i>	Pea leaf weevil
<i>Xyleborus dispar</i>	Pear blight beetle
Diptera (flies)	
<i>Anastrepha fraterculus</i>	South American fruit fly
<i>Ceratitis capitata</i>	Mediterranean fruit fly
<i>Dasineura mali</i>	Apple leaf curling midge
<i>Rhagoletis pomonella</i>	Apple maggot
Tephritidae	Tephritid fruit flies
Hemiptera	
<i>Anuraphis farfarae</i>	
<i>Aphis pomi</i>	Apple aphid
<i>Campylomma verbasci</i>	Mullein plant bug
<i>Dysaphis plantaginea</i>	Rosy apple aphid
<i>Eriosoma lanigerum</i>	Woolly apple aphid
<i>Edwardsiana crataegi</i>	
<i>Halyomorpha halys</i>	The brown marmorated stink bug
<i>Lepidosaphes ulmi</i>	Apple mussel scale
<i>Lygocoris pabulinus</i>	Common green capsid
<i>Lygus lineolaris</i>	Tarnished plant bug
<i>Parthenolecanium corni</i>	European fruit lecanium
<i>Psylla mali</i>	Apple sucker
<i>Quadraspidiotus ostreaeformis</i>	European fruit scale
<i>Rhopalosiphum insertum</i>	Apple-grass aphid
Hymenoptera (sawflies, wasps ants, bees)	
<i>Ametastegia glabrata</i>	Dock sawfly
<i>Hoplocampa testudinea</i>	Apple sawfly
Isopoda (woodlouse)	
Oniscidea spp.	
Lepidoptera (moths)	
<i>Adoxophyes orana</i>	Summer fruit tortrix
<i>Adoxophyes reticulana</i>	
<i>Amphipyra pyramidoides</i>	Humped green fruitworm
<i>Archips rosana</i>	Rose tortrix
<i>Archips podana</i>	Large fruit-tree tortrix
<i>Archips breviplicanus</i>	Asiatic leafroller
<i>Archips argyrospila</i>	Fruittree leafroller
<i>Clepsia spectrana</i>	Cabbage leafroller
<i>Coccus cossus</i>	Goat moth
<i>Coleophora hemerobiella</i>	Fruit tree case moth

<i>Cydia pomonella</i>	Codling moth
<i>Epiphyas postvittana</i>	Light brown apple moth
<i>Grapholita molesta</i>	Oriental fruit moth
<i>Lithophane antennata</i>	Widestriped green fruitworm
<i>Lacanobia subjuncta</i>	Lacanobia fruitworm
<i>Malacosoma Neustria</i>	Lackey moth
<i>Operophtera brumata</i>	Winter moth
<i>Orthosia incerta</i>	
<i>Orthosia</i> spp.	
<i>Phlogophora meticulosa</i>	Angle shades
<i>Phyllonorycter blancardella</i>	Spotted tentiform leafminer
<i>Phyllonorycter crataegella</i>	Apple blotch leafminer
<i>Phyllonorycter elmaella</i>	Western tentiform leafminer
<i>Spilonota ocellana</i>	Bud moth
<i>Stigmella malella</i>	Banded apple pigmy
<i>Stigmella incognitella</i>	Grey apple pigmy
<i>Synanthedon myopaeformis</i>	Red-belted clearwing
<i>Yponomeuta malinellus</i>	Apple ermine
<i>Zeuzera pyrina</i>	Leopard moth
Thysanoptera (thrips)	
Thripidae spp.	Thrips
Trombidiformes (mites)	
<i>Aculus schlechtendali</i>	Apple rust mite
<i>Eriophyes pyri</i>	Pearleaf blister mite
<i>Epitrimerus pyri</i>	Pear rust mite
<i>Panonychus ulmi</i>	European red mite
<i>Phyllocoptes malinus</i>	
<i>Tetranychus mcdanieli</i>	McDaniel spider mite
<i>Tetranychus urticae</i>	Twospotted spider mite
<i>Tetranychus viennensis</i>	Fruit-tree spider mite

Nematodes

Latin name	Common name (English)
<i>Meloidogyne arenaria</i>	
<i>Meloidogyne hapla</i>	
<i>Meloidogyne incognita</i>	
<i>Meloidogyne javanica</i>	
<i>Pratylenchus penetrans</i>	
<i>Pratylenchus vulnus</i>	
<i>Xiphinema americanum</i>	American dagger nematode
<i>Xiphinema rivesi</i>	

Sources: Grove et al. (2003), Betere Bomen (2018), UCANR (2018), Fruit pluktuin (2019), Groenkennisnet (2019).

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