

*FPS COST Action FP1202*

**Strengthening conservation:  
a key issue for adaptation of marginal/peripheral populations  
of forest trees to climate change in Europe (MaP-FGR)**

**SHORT TERM SCIENTIFIC MISSION REPORT**

***Evaluation of genetic erosion in European crab apple populations as a  
function of adaptation to environmental changes***

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## Abstract

Wild species have been exploited as donors of important traits to their cultivated relatives in various breeding programs. Over time diverse survey missions have been made to the areas of origin for collecting samples that will contain huge amount of genetic diversity to be used for novel needs. European wild apple (*Malus sylvestris* L.) is the only *Malus* wild species native to Europe growing on edges of forests; farmland hedges and marginal sites. Living in rather scattered meta-populations, they are exposed to genetic erosion in relation to underutilization, hybridization and habitat degradation in a sense of rapid climate change. In a literature review of the *Malus sylvestris* L. and other species studies we collected results describing gene diversity dynamics. Our aim was to perform comparative analysis of values of observed and expected heterozygosity with inbreeding coefficient as a measure of deviation in order to shape view about genetic erosion in European wild apple.

## 1 Introduction

European wild apple (*Malus sylvestris* L.) can be found across Western and Central Europe being important species for forest diversity. Representing also one of the apple wild relatives it is widely distributed across temperate areas in Eurasia where they grow in low-density populations in a wide range of habitats and environmental conditions (Cornille *et al.* 2014).

European wild apples are of high light requirement thus growing on edges of forests; farmland hedges and marginal sites on almost all soils (www.euforgen.org; Cornille *et al.* 2014). Besides changes in natural habitat (deforestation) (Jacques *et al.* 2009) and environmental conditions they are also suffering from lack of coppicing practice for rejuvenation of old trees. At the same time these populations are threatened by novel apple disease e.g. apple cancer and more specifically threatened by genetic erosion what partially may be caused by hybridization with domesticated apples followed by introgression. Different intermediate shapes are occurring but level of introgression is not precisely defined yet mainly due to the fact that those hybridization events are not recognized as threatening until recently. Trueness to type at the population level is highly correlated to the level of geographic isolation where more isolated populations showed less level of hybrids (Larsen *et al.* 2006)

A couple of decades ago a survey missions to explore apple wild relatives were initiated by different research groups in order to better decipher areal of growing and ecology of living crab apple populations in Europe. Crab apple was inventoried, collected and evaluated in several European countries and diverse studies are available about morphological and phenological variability of traits. Genetic diversity is assessed in Belgium (Jacques *et al.* 2009; Coart *et al.* 2003) and is concluded to be one of the most endangered tree species in the region. Similar work has been done in e.g. Denmark (Larsen *et al.* 2006), Germany (Reim *et al.* 2013); France/Germany (Schnitzler *et al.* 2014) or Poland where Czarna *et al.* (2013) were even telling about third

species – being a hybrid between *Malus sylvestris* L. and *Malus × domestica* L. (*Malus × oxyssepala*). Studies were done also outside Europe within in situ in the forest of Kazakhstan and Kyrgyz Republic and ex situ collections in USA (Gross *et al.* 2012). Cornille *et al.* (2013) analyzed *Malus sylvestris* accessions from next forests: Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Denmark, France, Germany, UK, Hungary, Italy, Norway, Poland, Romania, Spain and Ukraine and genetic profiles were obtained from 26 microsatellites. Velasco *et al.* (2010) tested *Malus* classification hypothesis by the analysis of molecular differences applying resequencing of 23 genes in 72 accessions of genus *Malus* representing 31 species where *Malus sylvestris* as well as *Malus × domestica* were also represented. Obtained results showed that *Malus sieversii* is possibly representing the same species as *Malus × domestica* while *Malus sylvestris* was placed in well separated clade. Coart *et al.* (2006) did chloroplast haplotyping joint to the microsatellite analysis and found molecular similarities between *Malus × domestica* and *Malus sylvestris*.

In recent times measuring or describing of genetic diversity is based on polymorphisms on DNA level expressed often through the observed and expected heterozygosity (gene diversity) as well as average number of alleles (allelic richness). Genetic potential and rich diversity is rather depicted in allelic richness and more rather in presence of population structure. E.g. allelic richness was slightly higher in wild apples when compared to the cultivated varieties in Danish and Iranian studies what might make a speculation about rather slow genetic loss (or increased level of hybridization followed by introgression).

Diverse researches are done on genetics and ecology of apple domestication and its impacts on wild apples. Hybridization is discovered to be major event in domestication of apple thus highlighting its value as an ideal model for unrevealing adaptive diversification processes in perennial fruit crops. Wild apple species display strong population structures and high levels of introgression from domesticated apple and this may threaten their genetic integrity (Cornille *et al.* 2014).

Lately hybridization has also been recognized as a severe threat to extinction of especially rare species being exposed to the more abundant species (Coart *et al.* 2003).

Genetic erosion is claimed to be on going in many crops with the special concern to their wild relatives. Often using argument is that varietal shift happened in favor of predominated modern bred hybrids. Old and traditional varieties are neglected and on margins of growing. Naturally growing populations like European crab apples are surviving on the edges of the forests in rather scattered populations. But what is indeed going on with the process that we are calling genetic? E.g. *Vitis vinifera* plants were dioecious before domestication thus obtaining a high level of diversity by outbreeding and this is in accordance with contemporary researches that grapevines showed tendency to a high degree of a heterozygosity in its genome (Aradhya *et al.* 2003; Lijavetzky *et al.* 2007; Salmasso *et al.* 2004). Apples might comprise similar benefits having different levels of self-incompatibilities on gametophytes level. Diverse *Malus* species therefore are hybridizing readily and cross-pollination between genetically different cultivars and species is essential for fruit production. Genetic diversity is regenerating by mutation over period of hundreds to millions of generations and measures of genetic diversity are required to document losses of genetic variation, evolutionary changes and genetic differentiation of populations. Heterozygosity and frequencies of alleles at individual loci are used to characterize genetic diversity in populations. Allele and genotype frequencies are in equilibrium under random mating when there are no other perturbing forces. Over all excess of heterozygous is than not necessarily characteristics of highly diverse population. Genetic potential and rich diversity is rather depicted in allelic richness and more rather in presence of population structure. Regarding wild population loss of genetic diversity might be attributed to inbreeding if on scene. Lower genetic diversity prevents those surviving environmental extremes and diseases outburst when compared to the populations with higher genetic diversity.

Available genetic diversity analysis on *Malus sylvestris* L. populations are comprising different number of samples and diverging genetic techniques. For evaluating the level of present diversity and their dynamics comparative analysis of different genetic parameters can be statistically assessed. After this kind of analysis

one can subjectively estimate genetic erosion mostly by comparing to the results obtained in other crops having similar biology as target crop.

## 2 Objective of the STSM

Our main objective was to consider the process of genetic erosion in European crab apple based on available studies from gene diversity analysis.

In order to formulate a conclusion we collected data describing gene diversity in *Malus sylvestris* L. from various studies and analyzed shifts in values. At the same time we compared them with equivalent values from different *Malus* species, other crops and their wild relatives.

Performed analysis might enable us to describe better state of genetic diversity in European wild apple populations. These analyses are valuable for monitoring the genetic loss in observed populations and for future forecasting analysis related to the dynamic environmental changes.

## 3 Links with Cost Action FP1202 MaP FGR

Due to its dualistic nature of being apple wild relative and important forest genetic resource there is growing need for extended knowledge of this source of diversity that can be used for breeding novel needs arising from environmental changes. At the same time these increasing needs are opening interest into evolutionary analysis particularly domestication events and solutions from molecular taxonomy. Only joint effort between forest and plant genetic resources communities may activate sustainable use and thus proper conservation of this important species being exposed to different threats.

## 4 Materials and methods

Values of observed ( $H_o$ ) and expected ( $H_E$ ) heterozygosity were collected by reviewing the publically available studies about *Malus sylvestris* L., *Malus sieversii* L. and *Malus × domestica* Borkh. (Table 1, 2 and 4). Diversity data from other wild species and fruit crops were also collected from the literature (Table 3 and 5). Our work is based on analysis of shifts of *Malus sylvestris* L. gene diversity along spatial gradients and is based on comparative studies where we contrasted equivalent genetic parameters from diverse researches and also different crops. As both values are calculated according to the formula already containing correction for sample size we were able to compare values from different studies without additional standardization. We calculated Inbreeding coefficient ( $F_{IS}$ ) from observed and expected heterozygosity according formulae  $F_{IS} = (H_E - H_o)/H_E$  in order to see the degree to which heterozygosity is reduced below the expectation.

## 5 Results

In different *Malus sylvestris* L. populations observed heterozygosity ranged from 0.636 in Arnsberg Gene Bank collection from Germany till 0.810 in Rhinau population from Schnitzler *et al.* (2014) (Table 1). Some of the analyzed populations had positive coefficient and being relatively high might point out to inbreeding but in certain cases can also be assigned to the low number of trees included into analysis. Negative values of  $F_{IS}$  might indicate outbreeding events and knowing that different *Malus* species hybridize readily outbreeding

might be expected at wider rate (Table 1). Mostly, expected heterozygosity was higher than observed and one can speculate that as not optimal predictive value for natural populations.

**Table 1** European crab apple *Malus sylvestris* obtained heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and inbreeding coefficient ( $F_{IS}$ ) in different populations obtained from various studies and ordered from smallest to highest values of  $F_{IS}$ . N represents the number of individuals sampled for analysis.

| Authors                       | Country/Region          | Population                         | N   | $H_o$ | $H_e$ | $F_{IS}$ |
|-------------------------------|-------------------------|------------------------------------|-----|-------|-------|----------|
| Schnitzler <i>et al.</i> 2014 | Germany / France        | Illkirch                           | 41  | 0.770 | 0.740 | -0.041   |
| Schnitzler <i>et al.</i> 2014 | Germany / France        | Erstein                            | 79  | 0.780 | 0.770 | -0.013   |
| Larsen <i>et al.</i> 2006     | Denmark                 | KA                                 | 50  | 0.754 | 0.746 | -0.011   |
| Larsen <i>et al.</i> 2006     | Denmark                 | Mean                               | *   | 0.778 | 0.770 | -0.010   |
| Larsen <i>et al.</i> 2006     | Denmark                 | NO                                 | 28  | 0.796 | 0.791 | -0.006   |
| Schnitzler <i>et al.</i> 2014 | Germany / France        | Daubensand                         | 3   | 0.780 | 0.780 | 0.000    |
| Schnitzler <i>et al.</i> 2014 | Germany / France        | Hardt                              | 73  | 0.780 | 0.790 | 0.013    |
| Schnitzler <i>et al.</i> 2014 | Germany / France        | Marckoisheim                       | 47  | 0.760 | 0.770 | 0.013    |
| Larsen <i>et al.</i> 2006     | Denmark                 | HE                                 | 50  | 0.794 | 0.815 | 0.026    |
| Larsen <i>et al.</i> 2006     | Denmark                 | KO                                 | 50  | 0.731 | 0.761 | 0.039    |
| Reim <i>et al.</i> 2013       | Germany                 | East Ore Mountains/14000ha         | 167 | 0.680 | 0.710 | 0.042    |
| Coart <i>et al.</i> 2003      | Belgium                 | Voeren/Belgimu (WBVo)              | 9   | 0.691 | 0.742 | 0.069    |
| Coart <i>et al.</i> 2003      | Germany                 | Arnsberg Gene Bank / Germany (WGF) | 6   | 0.636 | 0.703 | 0.095    |
| Coart <i>et al.</i> 2003      | Belgium                 | Viroin / Belgium (WBVi)            | 7   | 0.708 | 0.784 | 0.097    |
| Schnitzler <i>et al.</i> 2014 | Germany / France        | Rhinau                             | 3   | 0.810 | 0.900 | 0.100    |
| Coart <i>et al.</i> 2003      | Belgium                 | Meerdaal/Belgium (WBM)             | 28  | 0.706 | 0.810 | 0.128    |
| Cornille <i>et al.</i> 2012   | European countries (15) | -                                  | 40  | 0.750 | 0.870 | 0.138    |
| Coart <i>et al.</i> 2003      | Germany                 | Escherde Gene Bank / Germany (WGL) | 21  | 0.652 | 0.759 | 0.141    |

Same findings can be observed in populations of *Malus sieversii* L. (Table 2). With observed heterozygosity being higher than expected and always positive inbreeding coefficients, wild apples seemed to suffer from inbreeding depression.

**Table 2.** Wild apple *Malus sieversii* obtained heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and inbreeding coefficient ( $F_{IS}$ ) in different populations obtained from various published studies. N represents the number of individuals sampled for analysis and ordered from smallest to highest values of  $F_{IS}$ . N represents the number of individuals sampled for analysis.

| Authors                     | Country/Region                        | Population         | Species                | N   | $H_o$ | $H_e$ | $F_{IS}$ |
|-----------------------------|---------------------------------------|--------------------|------------------------|-----|-------|-------|----------|
| Omasheva <i>et al.</i> 2015 | Kazakhstan                            | Ketmen             | <i>Malus sieversii</i> | 8   | 0.705 | 0.701 | 0.007    |
| Omasheva <i>et al.</i> 2015 | Kazakhstan                            | Bel'bulak          | <i>Malus sieversii</i> | 36  | 0.771 | 0.787 | 0.019    |
| Omasheva <i>et al.</i> 2015 | Kazakhstan                            | Great Almaty gorge | <i>Malus sieversii</i> | 48  | 0.704 | 0.747 | 0.054    |
| Omasheva <i>et al.</i> 2015 | Kazakhstan                            | Almaty Reserve     | <i>Malus sieversii</i> | 48  | 0.707 | 0.753 | 0.061    |
| Richards <i>et al.</i> 2009 | Kazakhstan, Uzbekistan and Tajikistan | -                  | <i>Malus sieversii</i> | 949 | 0.693 | 0.749 | 0.075    |
| Omasheva <i>et al.</i> 2015 | Kazakhstan                            | Tau Turgen         | <i>Malus sieversii</i> | 40  | 0.631 | 0.716 | 0.115    |

Being values already corrected for sample size, observed and expected heterozygosity enabled us to compare data from *Malus* species to other wild species with similar reproductive biology. We collected data from various studies on wild grapevine (*Vitis sylvestris* L.) and wild olive (*Olea europea* var. *syvestris* L.) (Table 3). Values were in the same range like in *Malus* species and with significant differences in inbreeding coefficients particularly when compared to wild grapevine. Regarding wild grapevines, it seems that analyzed populations are endangered at higher extents and from both hybridization with perhaps its domesticated relative and inbreeding depression.

**Table 3.** Different wild species obtained heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ) and inbreeding coefficient ( $F_{IS}$ ) in different populations obtained from various published studies. N represents the number of individuals sampled for analysis and ordered from smallest to highest values of  $F_{IS}$ . N represents the number of individuals sampled for analysis.

| Authors                  | Country-Region/Population                     | Species                                   | N  | $H_o$ | $H_e$ | $F_{IS}$ |
|--------------------------|---|---|----|-------|-------|----------|
| Garfi <i>et al.</i> 2013 | Italy-Sicily/Riserva Boschi Favara e Granza   | <i>Vitis sylvestris</i>                   | 17 | 0.708 | 0.543 | -0.304   |
| Garfi <i>et al.</i> 2013 | Italy-Sicily/ Fiume Manghisi                  | <i>Vitis sylvestris</i>                   | 5  | 0.700 | 0.607 | -0.153   |
| Yoruk and Taskin. 2014   | Turkey-Marmara/ Marmara/Bursa                 | <i>Olea europea</i> var. <i>syvestris</i> | 37 | 0.480 | 0.450 | -0.067   |
| Garfi <i>et al.</i> 2013 | Italy-Sicily/ Riserva Pantalica e Valle Anapo | <i>Vitis sylvestris</i>                   | 18 | 0.778 | 0.761 | -0.022   |
| Yoruk and Taskin. 2014   | Turkey-Aegean/ Aegean/Manisa                  | <i>Olea europea</i> var. <i>syvestris</i> | 11 | 0.630 | 0.620 | -0.016   |
| Garfi <i>et al.</i> 2013 | Italy-Sicily/Riserva Zangara                  | <i>Vitis sylvestris</i>                   | 8  | 0.708 | 0.699 | -0.013   |

|                             |                                |  |      |       |       |       |
|-----------------------------|--------------------------------|--|------|-------|-------|-------|
| Yoruk and Taskin. 2014      | Turkey-Aegean/Aegean/Mugla     | <i>Olea europea</i> var. <i>sylvestris</i> | 7    | 0.520 | 0.520 | 0.000 |
| Garfi <i>et al.</i> 2013    | Italy-Sicily/Stretta di Longi  | <i>Vitis sylvestris</i>                    | 7    | 0.500 | 0.517 | 0.033 |
| Yoruk and Taskin. 2014      | Turkey-Mediterranean/Gaziantep | <i>Olea europea</i> var. <i>sylvestris</i> | 20   | 0.560 | 0.580 | 0.034 |
| Garfi <i>et al.</i> 2013    | Italy-Sicily/Fiume Sosio       | <i>Vitis sylvestris</i>                    | 13   | 0.667 | 0.691 | 0.035 |
| Yoruk and Taskin. 2014      | Turkey-Marmara/Balikesir       | <i>Olea europea</i> var. <i>sylvestris</i> | 26   | 0.590 | 0.630 | 0.063 |
| Garfi <i>et al.</i> 2013    | Italy-Sicily/Castelbuono       | <i>Vitis sylvestris</i>                    | 9    | 0.556 | 0.606 | 0.083 |
| Yoruk and Taskin. 2014      | Turkey-Mediterranean/Adana     | <i>Olea europea</i> var. <i>sylvestris</i> | 22   | 0.570 | 0.630 | 0.095 |
| Yoruk and Taskin. 2014      | Turkey-Mediterranean/Mersin    | <i>Olea europea</i> var. <i>sylvestris</i> | 32   | 0.540 | 0.610 | 0.115 |
| Yoruk and Taskin. 2014      | Turkey-Aegean/Izmir            | <i>Olea europea</i> var. <i>sylvestris</i> | 49   | 0.550 | 0.630 | 0.127 |
| Miyamoto <i>et al.</i> 2015 | Japan                          | <i>Cryptomeria japonica</i>                | 3781 | 0.801 | 0.923 | 0.132 |
| Garfi <i>et al.</i> 2013    | Italy-Sicily/Cava Sturia       | <i>Vitis sylvestris</i>                    | 4    | 0.542 | 0.625 | 0.133 |
| Bitz <i>et al.</i> 2015     | Germany/Hitch islands          | <i>Vitis sylvestris</i>                    | 25   | 0.464 | 0.693 | 0.330 |

Gross *et al.* (2014) investigated dynamics of genetic diversity in domesticated apple in comparison with other perennial and annual fruit crops. In their premises, the erosion of genetic diversity was expected in domesticated apple when compared to its wild relatives due to the bottlenecks during improvement processes. Their finding proved that domesticated apple retained 91.4% of the diversity present in wild relatives. Overall gene diversity among various apple cultivars (Table 4.) showed to be 0.791 that is similar to the value obtained by Gross *et al.* (2014) and when compared with other crops (Table 5) can be stated as high.

Overall gene diversity calculated from *Malus sylvestris* data was 0.741 and if compared to its domesticated relative can be stated as a high.

**Table 4.** *Malus × domestica* obtained heterozygosity ( $H_0$ ), expected heterozygosity ( $H_e$ ) and inbreeding coefficient ( $F_{IS}$ ) in different populations obtained from various published studies. N represents the number of individuals sampled for analysis and ordered from smallest to highest values of  $F_{IS}$ . N represents the number of individuals sampled for analysis.

| Authors                               | Country/Region                      | Population               | N   | Ho    | He    |
|---------------------------------------|-------------------------------------|--------------------------|-----|-------|-------|
| Gharghani <i>et al.</i> 2009          | Iranian landraces                   | <i>Malus × domestica</i> | 95  | 0.630 | 0.780 |
| Pina <i>et al.</i> 2014               | Spain/Aragon                        | <i>Malus × domestica</i> | 130 | 0.680 | 0.800 |
| Garkava-Gustavsson <i>et al.</i> 2013 | Sweden, Finland and EU              | <i>Malus × domestica</i> | 101 | 0.740 | 0.720 |
| Suprun <i>et al.</i> 2015             | Modern Russian apple tree cultivars | <i>Malus × domestica</i> | 31  | 0.786 | 0.755 |
| Bitz <i>et al.</i> 2016 unpublished   | Finland                             | <i>Malus × domestica</i> | 550 | 0.797 | 0.809 |
| Urrestarazu <i>et al.</i> 2012        | Spain                               | <i>Malus × domestica</i> | 259 | 0.800 | 0.820 |
| Liang <i>et al.</i> 2015              | Italy                               | <i>Malus × domestica</i> | 240 | 0.810 | 0.800 |
| Larsen <i>et al.</i> 2006             | Old apple cultivars                 | <i>Malus × domestica</i> | 29  | 0.821 | 0.846 |

**Table 5.** Different crops obtained heterozygosity ( $H_0$ ), expected heterozygosity ( $H_e$ ) and inbreeding coefficient ( $F_{IS}$ ) in different populations obtained from various published studies. N represents the number of individuals sampled for analysis and ordered from smallest to highest values of  $F_{IS}$ . N represents the number of individuals sampled for analysis.

| Authors                      | Country/Region  | Species                                  | Population                     | N    | Ho    | He    |
|------------------------------|-----------------|--|--------------------------------|------|-------|-------|
| Stajner <i>et al.</i> 2014   | Balkan          | <i>Vitis vinifera</i>                    | Balkan grapevine cultivars     | 140  | 0.775 | 0.712 |
| Miranda <i>et al.</i> 2010   | Spain           | <i>Pyrus communis</i> L.                 | Ancient Spanish pears          | 126  | 0.740 | 0.830 |
| Escribano <i>et al.</i> 2008 | Worldwide       | <i>Annona cherimola</i>                  | Cherimoya                      | 279  | 0.440 | 0.500 |
| Yoruk and Taskin. 2014       | Turkey          | <i>Olea europea</i> var. <i>europaea</i> | Cultivated olive               | 26   | 0.480 | 0.570 |
| De Souza <i>et al.</i> 2015  | Peru and Brasil | <i>Hevea brasiliensis</i>                | Rubber tree                    | 1095 | 0.640 | 0.760 |
| Bitz <i>et al.</i> 2015      | Germany         | <i>Vitis vinifera</i>                    | Worldwide cultivars collection | 60   | 0.644 | 0.826 |

## 6 Discussion and conclusion

Biological diversity is not only dependent on the relationships between species but also on the way in which the species are partitioned by the extinction pattern. Solow *et al.* (1993) focused on the measurement of biological diversity not considering the value of biological diversity. They are concluding that in practice conservation decisions need to weigh the value of biological diversity along with other values accruing to the plant and animal species (e.g., use value, existence value, etc.). **Although obtaining relatively high gene diversity values in European crab apple we are cautious by making a conclusion about absence of genetic erosion within this wild species. One of possible conclusions can be that high gene diversity within *Malus sylvestris* is maintained due to the permanent hybridization with other *Malus* species most particularly with**

**escaped edible cultivars due to the self-incompatibility and open pollination.** In general they are living in very scattered populations, very often as isolated trees and habitat degradation is permanent threat to its maintenance. Overall conclusion could be that interspecific hybridization (or co-existence) might be responsible for maintenance of relatively high level of gene diversity within European wild apple. At the same time is a threat to species authenticity. Also high degree of polymorphism is ensured by biology of species itself that it is predetermined to collected high levels of variability being often self-incompatible thus readily hybridizing with other *Malus* species.

Its economical insignificance and inconspicuous appearance in combination with present day intensive forest and agricultural management systems induced a further decrease of this species. As a result, fragmentation and spatial isolation of suitable habitats may have led to a reproductive separation of long-established meta-population networks, even in species adapted to naturally scattered distributions and small population sizes (Wagner 2006).

Wild apple species are important as valuable sources of diversity for apple breeding for novel needs. Crab apple researches are also needed for understanding fruit tree evolution having genus *Malus* as a model system (Cornille *et al.* 2014). Well defined program for conservation is also needed in order to secure future evolution of the genetic resources (Eriksson *et al.* 1995). Genetic diversity of wild populations should be maintained in wide range to ensure natural selection for enabling adaptations to local environment over time (Larson *et al.* 2006). Proper maintenance should be proceeded on to minimize genetic erosion of this material whose diversity is important for breeding apples for the future needs.