Hungarian University of Agriculture and Life Sciences

Factors affecting the distribution and damage of economically important aphid species in apple and apricot orchards

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Csaba Borbély

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Doctoral School

Name: PhD School of Horticultural Science

Scientific branches: Agricultural Sciences (Agriculture and Horticulture)

Leader: Prof. Dr. Éva Zámbori-Németh

Professor

Hungarian University of Agriculture and Life Sciences,

Institute of Horticultural Sciences

Supervisor: Prof. Dr. Viktor Markó

Professor

Hungarian University of Agriculture and Life Sciences,

Institute of Plant Protection

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Approval of the leader Approval of the supervisor

1. Background of the work, objectives

The green apple aphid (*Aphis pomi* De Geer) and the spirea aphid (*Aphis spiraecola* Patch) are common and important pests of apple in Europe and in many apple-growing regions of the world. The damage caused by the two species is identical: slight malformations of leaves, shoot distortion, reduced shoot growth, reduced chlorophyll content of leaves, and assimilate accumulation of the plant, but direct fruit damage can also occur in the presence of large aphid populations (Varn and Pfeiffer, 1989; Kaakeh et al. 1992, 1993). Nonetheless, the origin and biological parameters of the two species differ greatly. *A. pomi* is an aphid species of Palaearctic origin, native to Europe (Baker and Turner, 1916), while *A. spiraecola*, a sub-tropical aphid species of East Asian origin, has been spreading from south to north as an invasive species in Europe in recent decades (Rakauskas et al. 2015; CABI, 2023). The life cycle parameters of the two species also differ significantly: the oligophagous aphid *A. pomi* spends its entire life cycle on Maloideae host plants including apple (Baker and Turner, 1916), while *A. spiraecola* is a polyphagous, host-alternating species whose winter host plants, under temperate climate conditions, are *Spirea* species, while apple is one of its numerous summer hosts (Blackman and Eastop, 2010). However, some authors have also observed the ability of *A. spiraecola* to overwinter on apple as a primary host in several parts of the world (Miller et al. 1926; Komazaki, 1982; Pfeiffer et al. 1989). In North America, following the appearance of *A. spiraecola*, the relative abundance of the previously widespread *A. pomi* in the green aphid complex declined rapidly within a few years, with a shift in dominance between the two species (Pfeiffer et al. 1989; Brown et al. 1995; Mayer and Lunden, 1996). One of the reasons for the shift in dominance could be the better adaptability and higher tolerance to pesticides of *A. spiraecola* and its ability to overwinter on apple, as observed by several authors (Hogmire et al. 1992; Lowery et al. 2006).

In Hungary, both *A. pomi* and *A. spiraecola* form a mutualistic association with the black garden ant (*Lasius niger* L.). In return for the carbohydrate source provided by aphids, the ants protect the aphid colonies from various natural enemies and also play an important role in maintaining the colony's hygiene (Delabie, 2001). If this mutualistic relationship would be disrupted in some way (e.g., by physically excluding ants or by distracting them with some chemicals), the number of aphids can be significantly reduced by increased predation pressure on the colonies (Nagy et al. 2013; 2015).

Similar to *A. spiraecola*, the apricot aphid (*Myzus mumecola* Matsumura), a new aphid species with East Asian origin, is also spreading in European apricot growing regions. The new pest was found for the first time in Europe in Italy in 2016, where it caused severe damage to the apricot orchards that had not been seen before (Panini et al. 2017). Very little is known about the distribution, the life history and the damage of this aphid species in Europe. Thus, given the importance of apricot as a fruit crop, there is need for more research attention in this area.

The major focus areas of my research are as follows:

- Survey of the distribution of the spirea aphid (*A. spiraecola*) on different host plants in Europe,
- Revealing the overwintering strategy of the spirea aphid (*A. spiraecola*) in Hungary,
- Disentangle the effects of climatic factors, shoot growth and orchard management systems (organic versus conventional) on species composition, abundance, and fitness of green aphid assemblages in apple orchards in Europe,

- Testing of the efficacy of two environmentally friendly sugar feeding methods (bottle feeders and agar jelly cubes) in disruption of ant-aphid interactions and for the control of green aphid assemblages on apples in an organic apple orchard,
- Detection of the occurrence of the apricot aphid (*M. mumecola*) in Hungary, assessment of its life history and the susceptibility of certain apricot varieties to the pest and detection of the *Plum Pox Virus* (PPV), a significant pathogen of stone fruit trees, from the body of the pest.

2. Materials and methods

2.1. Distribution of *Aphis spiraecola* in Europe

The faunal survey of *A. spiraecola* was carried out in five European countries (Kosovo, Slovakia, the Czech Republic, the United Kingdom, Denmark) by aphid sample collections on different host plants throughout the growing seasons for three years. The collected aphids were identified morphologically and molecularly (based on the mitochondrial cytochrome c oxidase COI fragment).

2.2. Overwintering strategy of *Aphis spiraecola* in Hungary

The ability of *A. spiraecola* to overwinter on apple as a primary host plant was examined by collecting samples at ten sites of Hungary in April of seven years. During the sampling, only stem mothers and adults of the first generation of daughters were collected, and the aphids were identified morphologically.

2.3. Species composition, abundance and fitness of the green aphid assemblage in European apple orchards

The survey was carried out in 44 apple orchards across twelve regions (ten countries) of Europe. In each region, 2 pairs of apple orchards were sampled, one of which was always a conventional orchard manly under integrated pest management, and the other an organically managed orchard.

The selected orchards were sampled four times a year, in May, June, July and September, across two growing seasons (2018 and 2019). We sampled ten shoot tips of ten randomly selected trees to assess the intensity of host plant growth, and then estimated the abundance of green aphids in each orchard at three randomly selected shoot tips of the ten trees. Sampling was carried out from 33 randomly selected aphid colonies per orchard by collecting 3 (colony size < 100 individuals) or 20 (colony size ≥ 100 individuals) adult wingless aphids per colony. The aphids were identified morphologically and the length of their hind tibia was measured as a fitness parameter. Sampling date, the bioclimatic region of the orchard [for which we used two stratification models that divide Europe into bioclimatic zones: Metzger et al. (2005) and Botti (2018)] and the pest management strategy of the orchard (conventional versus organic) were recorded and used as explanatory variables.

The statistical analysis of the data was carried out using general mixed-effects and linear mixed-effects models.

2.4. The sugar feeding of ants for control green apple aphids

The study was carried out in an organic apple orchard in Újfehértó, Hungary, during two growing seasons (2014 and 2015) on 'Prima' and 'Remo' cultivars. Prior to the study, the presence of ant workers was assessed in the selected blocks of the study consisted of two adjacent apple trees.

In the study, the efficacy of the two sugar feeding methods, a bottle self-feeder filled with 30 m/V% sucrose solution and an agar jelly cube cooked from 30 m/V% sucrose solution and placed on the trunk of the trees was compared with untreated control blocks. Green aphids were naturally spreading in the experimental orchard, and aphid abundance, the number of ants in the aphid colonies and on the feeders, and the number of natural enemies in the aphid colonies were recorded in the blocks with each treatment on two occasions (June and July) in both years of the study, counted the number of ants and natural enemies, both day and night.

The effects of each treatment on the abundance of aphids, ant workers and natural enemies were compared using multi-factor analysis of variance and Games-Howell post hoc tests.

2.5. The occurrence, life history and damage of the apricot aphid (*Myzus mumecola*) in Hungary

During the first mass outbreak of the pest in Hungary (spring 2020), we collected samples from aphid colonies from different regions of the country, and the species was identified morphologically and molecularly (COI fragment). From the end of March to the end of June, we monitored the development of the pest, included the appearance of the different forms and the damage caused on 18 selected apricot trees. In July 2020, the susceptibility of each apricot variety to the pest was compared on the basis of damage symptoms on 13 varieties of five apricot orchards in Hungary. Molecularly, using two-step PCR and a PPV-specific primer pair, we attempted to detect the presence of PPV virus in the bodies of previously collected aphids, and enzymatic restriction was used to identify the specific strain of the virus detected.

3. Results and discussion

3.1. Distribution of *Aphis spiraecola* in Europe

Aphis spiraecola was detected as a new species for the fauna in Kosovo (apple; 2 sites), Slovakia (apple, quince, Vanhoutte spirea; 4 sites), the Czech Republic (apple; 2 sites) and Denmark (apple; 1 site), while stable presence and colony formation of the species on apple, quince, pear, and firethorn was confirmed in the UK (6 sites). Comparing our results with former studies (Rakauskas et al. 2015; CABI, 2023), we found that *A. spiraecola* is now a widespread species throughout Europe and can be expected to occur in any region of the continent.

3.2. Overwintering strategy of *Aphis spiraecola* in Hungary

In this study, we collected 1126 aphid individuals from a total of 114 colonies (stem mothers and the first generation of daughters), all belonging to the species *A. pomi*, i.e., the overwintering ability of *A. spiraecola* on apples reported by other authors (Miller et al. 1926; Komazaki, 1982; Pfeiffer et al. 1989) was not observed in Central Europe.

One possible explanation for the differences between our findings and those reported in Eastern Asia and the United States may be that *A. spiraecola* has different host-specific biotypes, which are more or less adapted to one of the potential winter hosts and also show some differences in life history (Komazaki, 1983; 1990; 1991; 1998). The presence of an apple-specific biotype among others in East Asia and the United States, and its absence in Europe, could easily explain the lack of *A. spiraecola* populations overwintering on apple in Europe.

It is also possible that following the classical host-alternating life cycle is simply more beneficial for *A. spiraecola* than following a non-host-alternating strategy under the climate conditions in Central Europe.

3.3. The species composition, abundance and fitness of the green aphid assemblage in European apple orchards

Our results showed that the proportion of *A. spiraecola* and *A. pomi* was significantly influenced by the climate zone of the orchard: in both climate classification models, *A. spiraecola* was the dominant species in warm and dry climate zones, while *A. pomi* was the predominant species in cool and humid climate zones. Between these two extremes, the proportion of *A. spiraecola* showed a decreasing trend with a high fluctuation, i.e., in these regions, albeit with different probabilities, either one or the other species could become dominant in some years or orchards.

Distribution map of the relative abundance (proportion) of the two species in Europe (Fig. 1) shows that the proportion of *A. spiraecola* is strongly decreasing, while the proportion of *A. pomi* is strongly increasing along a south to north gradient. *A. spiraecola* became dominant over *A. pomi* only in the southern, low-altitude regions of Europe, while the latter maintained its dominance in Central and Northern Europe, i.e., a shift in dominance similar to that in North America did not occur in Europe. This could be explained by the different origins of the two species (*A. pomi* is native to Europe), their differing lifestyles on the two continents (e.g. *A. spiraecola* does not overwinter on apples in Europe) or the different climatic parameters of the two continents, which may result in *A. spiraecola* behaving as a subtropical species and only being able to significantly increase its populations in certain regions of the continent under certain conditions (Hullé et al. 2010). The differences in the proportions of the two species between the two years were probably due to the drought stress that cumulated over the two years (Hari et al. 2020) or could be linked to the North Atlantic Oscillation (Climate Compact Company, 2023).

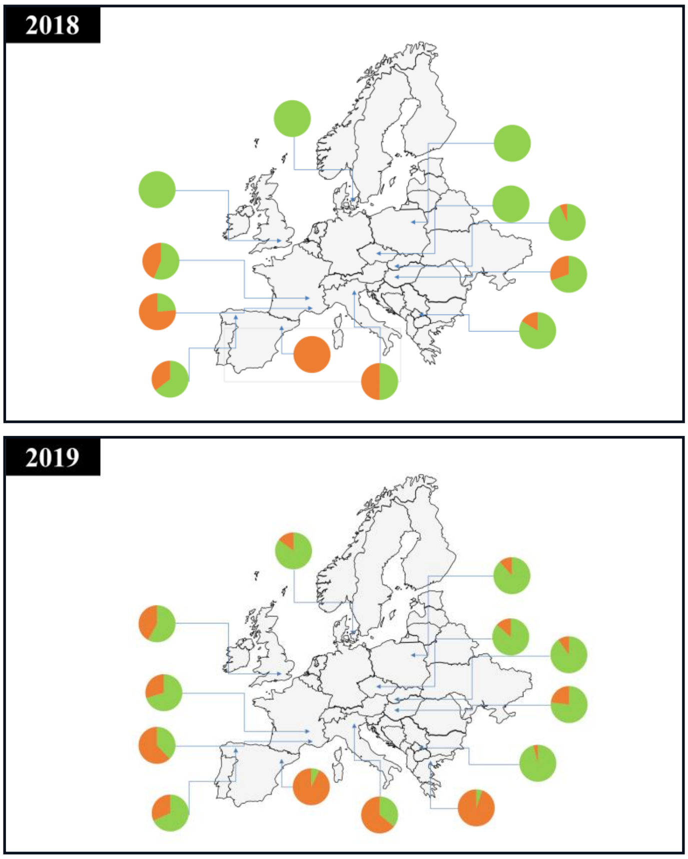

Fig. 1. Distribution and dominance of *A. spiraecola* and *A. pomi* in European apple orchards in 2018 and 2019 (green: *A. pomi*, orange: *A. spiraecola*)

The average percentage of growing shoots in the studied orchards decreased significantly during the growing season and was closely followed by a decrease in the probability of occurrence and length of hind tibiae for both aphid species. For both species, the highest probability of occurrence was in May, while the peak abundance occurred one month later in June. The observed pattern is consistent with the life cycles of both species in Europe and also indicates that the performance of both species is highly dependent on the growth vigour of the host plant, i.e., our findings do not confirm the higher tolerance of *A. spiraecola* to reduced shoot growth observed by some authors (Komazaki, 1988; Gao et al. 2020). The one-month delay in the maximum abundance may be explained by the population increase period of aphids following the immigration in May (Komazaki, 1988).

When comparing the effects of the two pest management strategies (conventional versus organic), we found no evidence that *A. spiraecola* is more tolerant to insecticide compounds widely used in Europe (Hogmire et al. 1992; Lowery et al. 2006) than *A. pomi*, which could be explained by the different reproductive behaviour of the two species in Europe (susceptible strains of *A. spiraecola* may survive on alternative host plants), their different genetic backgrounds, or to less favourable environmental conditions for *A. spiraecola* in Europe.

2.4. The sugar feeding of ants for control green apple aphids

Both bottle-feeders and sugar-feeding with agar jelly cubes significantly reduced the number of ant workers on aphid colonies and, as a consequence, the number of aphids in the tested orchard in both years (Figure 2). Although the low number of the natural enemy individuals recorded in aphid colonies did not allow a statistical evaluation of the data on species level, statistical analysis of the cumulated natural enemy data showed that the improved aphid control by the treatments was a consequence of higher predation pressure on aphids.

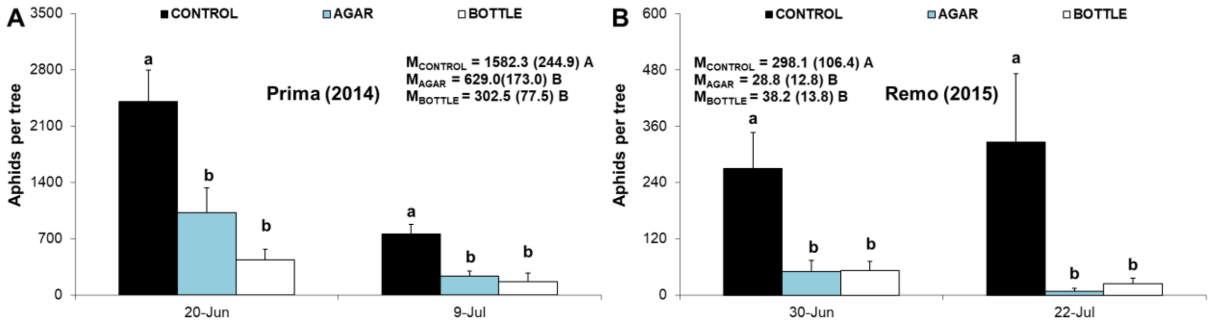

Fig. 2. Numbers of green aphids (*Aphis* spp.) on trees treated with each type of feeders (A) in 2014 and (B) in 2015. $M_{CONTROL}$, M_{AGAR} and M_{BOTTLE} show the mean (± standard error) values of the Repeated Measures ANOVA. Different letters indicate different levels of significance (lower case: comparison by samplings, upper case: ANOVA).

Our results confirm that the feeding method tested by Nagy et al. (2013; 2015) on *Dysaphis* species and further developed by us was effective in the disruption of the *L. niger*-*Aphis* spp. relationship, but further improvements of the method (addition of attractants, simpler and more stable application) are needed before its introduction for commercial use.

3.5. The occurrence, life history and damage of the apricot aphid (*Myzus mumecola*) in Hungary

We collected *M. mumecola* individuals from six localities in different regions of Hungary (Győr, Győrszentiván, Balatonalmádi, Budapest-Budafok, Pomáz, Gönc), and confirmed the presence of the species in Szentkirályszabadja, Érd, Törökbálint and Budapest-Soroksár based on the typical symptoms of the pest caused on apricot. We were the second in Europe after Italy (Panini et al. 2017) and the first in Hungary to report the occurrence of *M. mumecola*, and our results suggest that the species became a widespread and common pest of apricot in Hungary.

Based on our observations, *M. mumecola* follows a classical host-alternating life cycle in Hungary, although its summer host is still undiscovered. The new pest, in contrast to aphid species that were reported from apricot trees only occasionally (Pénzes et al. 2003), causes serious damage: its feeding from the phloem causes severe leaf distortion, premature leaf drop, shoots curl, while the shoot apex often necroses and re-sprouts, thus encouraging unwanted branching. The studied apricot varieties were classified into three groups (tolerant, moderately tolerant, susceptible) based on the severity of damage symptoms, these differences are presumably due to physiological or genetic characteristics of each variety (Dogimont et al. 2010).

Strain D of PPV, the most important viral disease of apricot, was detected from the bodies of the *M. mumecola* specimens we collected, so the role of this pest as a viral vector is likely to be increasingly important in the future (Kimura et al. 2016).

4. Conclusions and suggestions

Our final conclusion is that the invasive species introduced to Hungary and Europe, including *A. spiraecola* and *M. mumecola*, are associated with a complex ecosystem in their new habitat. The number of influencing factors in this ecosystem is considerably greater than the number of factors analysed in our studies. Our results show, however, that changes in a few factors (e.g., the geographical location of the habitat, bioclimatic factors, nutrient quality, pest management strategy, disturbance of mutualistic ant partners, or even the change of the host plant) can significantly affect the performance of a species and thus its damage.

A. spiraecola has become a common aphid species in apple orchards across Europe, but the shift in dominance seen in North America has only occurred in the southern part of the continent, so control of *A. pomi* remains the backbone of the control of green aphids in apple in Europe. However, the different life strategies of the two species in Europe need to be taken into consideration when designing pest control strategies: some of the technologies (e.g. oil spraying at the end of winter) that are effective against *A. pomi*, which spends its full life cycle in the apple orchards, are not effective against host-alternating *A. spiraecola*, while the non-host alternating life cycle increases the probability of emergence of pesticide-resistant populations of *A. pomi*.

Both the bottle feeders and agar jelly cubes we used proved to be effective in the ecological control of green aphids on apple. Both methods can be used without any modification to protect apple trees in home gardens, but their applicability for large-scale commercial use requires further development (cheaper and sustainable production, simpler and faster treatment).

The appearance of the apricot aphid in Hungary has led to a change in the pest management strategy of apricot: in the spring, during the emergence of the stem mothers, it became necessary to introduce an insecticide treatment (authorised active substances in Hungary: lambda-cyhalothrin, deltamethrin, acetamiprid, spirotetramat) to prevent direct damage caused by the pest. The expansion of the species in Europe is currently underway, and it is likely to cause serious damage in the near future in all apricot-growing regions of the continent.

5. New scientific results

The main findings of my research work are as follows:

- As a new record to the aphid fauna, we were the first to report the presence of *A. spiraecola* from Denmark, the Czech Republic, Slovakia, and Kosovo, while in the UK we have confirmed the stable presence of the species in several regions of the country on different host plants.
- We investigated the overwintering strategy of *A. pomi* and *A. spiraecola* in Hungary through seven years of sampling in different locations of the country and confirmed that *A. spiraecola* does not or only to a minor extent overwinters on apple as a primary

host in Hungary, thus in Central Europe it follows a classical host-alternating lifestyle with *Spiraea* spp. as primary winter host.

- We assessed the dominance of *A. pomi* and *A. spiraecola* in apple orchards of different climatic and geographical locations in Europe by sampling in 12 geographical regions of the continent (10 countries, 44 apple orchards). *Aphis spiraecola* became dominant over *A. pomi* only in the Southern regions of Europe, while *A. pomi* remained the dominant species of the complex in the further regions, with great dominance in the Northern and Western Europe. The climate zone of the orchards significantly influenced the ratio of the two species with a great dominance of *A. spiraecola* under arid and high-temperature climate, and *A. pomi* under humid and lower temperature climate, while in the transitional zones, both species can have outbreaks in some years and orchards.
- We have revealed the effects of the intensity of the shoot growth of the host plant, the growing season (time of sampling) and the pest management strategy of the orchard on the probability of occurrence, abundance, proportion and fitness of the two species. The probability of presence and fitness of both species showed a decreasing trend during the growing season, while the maximal abundance of both species was recorded in June. The ratio of *A. spiraecola* showed a decreasing trend from May to September. The cessation of shoot growth caused a decrease in the performance of both species at the same level. The expected higher performance of *A. spiraecola* compared to *A. pomi* under higher insecticide pressure was not observed in Europe.
- A test in an organic apple orchard demonstrated the effectiveness of feeding ants with sucrose solution as an environmentally friendly control method for *Aphis* spp. control based on disruption of ant-aphid interactions and thus increased predation pressure on aphid colonies. Of the two types of feeders we developed, we confirmed the better efficacy of the bottle feeder over the agar jelly cube in controlling aphid populations.
- We confirmed the appearance of *M. mumecola* as a new species for the aphid fauna of Hungary, second country in Europe after Italy. Descriptive observation was carried out to reveal the life cycle of the species and the damage caused by it, and the susceptibility of different apricot cultivars grown in Hungary to the new pest was compared. PPV virus (strain D) was detected from the body of all individuals studied.

6. List of publications

My research resulted resulted in the following scientific publications by the completion of the manuscript (27.08.2023):

Publication in a peer-reviewed journal with impact factor:

- BORBÉLY, C., GYÖRGY, Z., JACOBSEN, S. K., MUSA, F., OUŘEDNÍČKOVÁ, J., SIGSGAARD, L., SKALSKÝ M., MARKÓ, V. (2020): First records of the invasive aphid species, *Aphis spiraecola*, in Kosovo, Slovakia, the Czech Republic, the United Kingdom and Denmark. *Plant Protection Science* 57 (1) 70–74. pp. (Q2)
- BORBÉLY, C., GYÖRGY, Z., SZATHMÁRY, E., MARKÓ, V. (2021): Apricot aphid, *Myzus mumecola* (Matsumura), a new and important pest of apricot in Hungary. *Journal of Plant Diseases and Protection* 128 (3) 781–787. pp. (Q2)
- BORBÉLY, C., NAGY, C. (2022): Providing sugar sources for ants improves the biological control of *Aphis* spp. in apple orchards. *Biological Control* 175, 105056. (Q1)

Publication in a peer-reviewed journal:

- BORBÉLY, C., GYÖRGY, Z., SZATHMÁRY, E., MARKÓ, V. (2021): Kajszilevéltetű (Myzus mumecola) – új kártevőfaj a hazai kajsziültetvényekben. Növényvédelem 82 (5) 193–200. pp.

Publication in conference proceedings:

- BORBÉLY, C. (2018): Helyzetértékelés a zöld gyöngyvessző levéltetű (*Aphis spiraecola* Patch; Hemiptera: Aphididae) jelentőségéről hazai almaültetvényekben. *Ifjú Tehetségek Találkozója, Szent István Egyetem, Budapest, 2018. december 7. Konferenciakiadvány*, 13–16. pp. (full paper)
- BORBÉLY, C., MARKÓ, G., LADÁNYI, M., MARKÓ, V. (2022): Analysis of the competition between aphid species *Aphis pomi* De Geer and *Aphis spiraecola* Patch. *Alternative Strategies of Plant Protection Against Invasive Insect Pests, Book of Abstracts,* 12. pp. (absztrakt)

Other scientific publication:

- BORBÉLY, C., SZATHMÁRY, E., MARKÓ, V. (2022): Megjelent hazánkban a kajszilevéltetű, a szilvahimlő vírus vektora. Mit tehetnek a kajszitermesztők? *Agrofórum Extra* 95, 30–33. pp.

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