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# Determination of Frost Resistance of Sweet Cherry Varieties Using Laboratory Freezing

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**ABSTRACT**
The article presents the study results of the frost resistance of sweet cherry varieties of different ripening periods under artificial freezing. The damage degree of various tissues of one- and two-year-old wood, as well as fruit formations, was analyzed. In the group of early-ripening and late-ripening varieties, a significant resistance of the bark to the effect of low temperature was found, the average damage score was 0.7. Among late-ripening cherry varieties Drogana yellow had low-temperature damage to one-year-old wood, while no negative effect on the wood was observed for the Amazonka variety. The highest overall degree of freezing of one-year wood was observed for the varieties Mliivska yellow and Drogana yellow, the lowest values were obtained for the varieties Meotida, Mirage and Amazonka. Dispersion analysis based on a combination of factors confirmed the highest degree of wood freezing for the Mliivska yellow and Drogana yellow varieties. Among the early-ripening varieties, the two-year-old wood froze the most in trees of the Dar Mlieva variety, where the highest degree of damage to the tissues of the bark, cambium, and wood was observed. The least tissue damage was in the Mliivska yellow variety, however, its core had the maximum damage not only within the group, but also among all the studied varieties. By artificial freezing in laboratory conditions, it was established that the highest frost resistance of annual and perennial wood is characteristic of the early ripening variety Zoryana, medium ripening Meotida and late ripening Amazonka. As a result of laboratory freezing, the frost resistance of fruiting bodies did not exceed 2.2 points. The fruiting formations of the Drogana yellow exceeded all the studied varieties in terms of the level of damage. High resistance to low temperatures was shown by the fruiting formations of the varieties Zoryana, Mirage and, especially, Alyonushka. The damage level of other pomological varieties was in the range of 1.9–2.25 points. The most negative effect of low temperatures was caused on flower germs.

**Keywords:** sweet cherry varieties, frost resistance, damage to shoot parts, artificial freezing.

## INTRODUCTION

Winter and frost resistance of cherries are important features that determine the limits of distribution of the variety and its industrial value. High or low winter resistance of plants depends not only on the climatic conditions of the cultivation place, the weather conditions of the growing season, but also on varietal characteristics.

Spring late frosts can also be a risk for production, both in regions with a moderate and in regions with a cold climate. Cold temperatures in spring following high temperatures in late winter and early spring are one cause of economic losses. Bacterial cancer infection often develops in places damaged by low temperatures. Thus, not only the annual harvest is lost, but also the health of the trees. Determining of the frost damage to a tree is difficult because it depends on many factors, such as variety characteritics, intensity of pruning, duration of exposure to low temperature, speed of freezing/thawing, resting stages of the tree during freezing [Chmielewski et al, 2018; Dziedzic et al., 2019]. However, according to a number of scientists [Kaya et al, 2021; Cittadini et al., 2006; Shubenko, 2018] the cold resistance of cherry and sour cherry varieties is genetically determined and varies between varieties.

Sudden low temperatures can occur in the spring, when some cherry varieties are more susceptible to frostbite and flower bud damage. From year to year, these are delicate moments for producers. Differences in cold resistance and lethal temperatures were observed during the same phenological stage (bud opening) for the same variety, as well as among the tested varieties (Katalin, Kordia, Burlat, Ryvan and Regina) [Ozherelieva et al, 2020; Asănică et al., 2014]. To determine the frost resistance of buds, next freezing modes was used: -7 °C/0.5h; -7 °C/1h; -1.5 °C/0.5h; -1.5 °C/1h; -2.5 °C/ 1 hour. The observed damage strongly depended on the stage of flower bud development. A progressive vulnerability of the bud to low temperatures was observed. Cultivars were sometimes observed to be susceptible, semi-susceptible or hardy, this feature varying with the sum of active temperatures above 7 °C. The maximum winter hardiness of cherries is a genetically fixed feature, but changes in this property may occur due to climatic influences, so additional years of observation are necessary [Chitu et al, 2006; Chmielewski et al., 2017; Feldman et al., 2016].

Selection of rootstocks can also be effective in preventing frost damage, mainly by anticipating or delaying the flowering time of the variety [Kishchak, 2011]. In addition, rootstocks can directly affect resistance to frost damage. For example, it is believed that the most cold-resistant are cherry seedlings of the “Mahaleb” variety [Narandžić et al, 2021; Miranda et al., 2005].

Frosts, which occur from time to time in nature, make it possible to evaluate varieties and rootstocks for cold resistance. Artificial freezing tests are also conducted in the laboratory to determine the resistance to frost damage of various varieties [Kaya et al, 2022; Fadón et al, 2017]. However, there are not many papers on the assessment of the percentage of damage of cherry varieties by natural frosts.

Evaluation of frost resistance by the field method requires a long period, until a severe winter comes and differences in plant resistance become apparent [Herrero et al, 2017]. The laboratory method allows to speed up the evaluation process.

## RESEARCH CONDITIONS AND METHODS

The objects of research were sweet cherry varieties of different ripening periods of the breeding by the Mliiv Institute of Horticulture named after L.P. Symyrenko of the National Academy of Agrarian Sciences (NAAS) of Ukraine (Aborygenka, Biryuza, Dar Mliyeva, Zoryana, Mliivska yellow), the Institute of Irrigated Horticulture of the NAAS (Mirage, Melitopolska krapchasta, Meotida), the former Donetsk research station of the Institute of Horticulture of the NAAS (Donetski Ugolyok, Alyonushka, Amazonka). The research was carried out in the cherry plantations of the Nemyrivska varietal testing station of the Forest-Steppe zone of Ukraine.

Laboratory freezing was carried out in a “Grunland” type freezer. The maximum degree of freezing was estimated at 5 points. To determine the frost resistance of plants, plant samples were frozen in the following mode: in January–February, the freezing temperature limits were –25, –30, and –35 °C; in the first half of March limits were –20, –25 and –30 °C at a rate of temperature decrease of 5 °C per hour and holding the set temperature for 4-6 hours.

To determine the stability of plants after freezing, anatomical sections of the samples were performed, followed by microscopic analysis of individual tissue damage and scoring. Sampling of one- and two-year-old wood was carried out a week before the agreed term of freezing [Bublyk et al, 2013].

## RESULTS AND DISCUSSION

Laboratory freezing showed that early ripening varieties were most damaged by low temperatures (on average 2.78 points), especially Mliivska yellow, 3.52 points (Table 1). Among them, the Alyonushka variety stood out with a minimum value of 1.14 points. With the exception of Alyonushka and Mirage varieties, the apical buds had the maximum level of damage (about 5 points in most cases). Two-year-old wood was more resistant to the effects of low temperatures, where the average damage among varieties was 1.23 points.

The reproductive buds of the early-ripening variety Mliivska yellow and the late-ripening Biryuza were the most damaged, while the mid-ripening varieties were the least affected by the

**Table 1.** Damage to parts of sweet cherry branches after laboratory freezing at a temperature of minus 30 °C, points

| Pomological variety | Branch parts | | | | |
|---|---|---|---|---|---|
| | apical bud | reproductive bud of a one-year shoot | reproductive bud of fruit formation | 1-year-old wood | 2-year-old wood |
| Early ripening varieties | | | | | |
| Dar Mliyeva | 4.8 | 2.6 | 2.5 | 2.1 | 2.3 |
| Zoryana (k) | 4.5 | 2.1 | 0.7 | 1.7 | 0.9 |
| Mliivska yellow | 4.8 | 4.3 | 2.7 | 3.9 | 1.9 |
| Medium ripening varieties | | | | | |
| Mirage | 3.3 | 1.0 | 2.4 | 0.8 | 1.5 |
| Alyonushka | 1.8 | 1.3 | 0.9 | 1.1 | 0.6 |
| Aborygenka | 5.0 | 2.1 | 1.0 | 2.6 | 1.1 |
| Melitopolska krapchasta | 4.5 | 1.4 | 1.5 | 2.5 | 2.8 |
| Meotida (k) | 5.0 | 1.3 | 1.9 | 0.5 | 0.6 |
| Late ripening varieties | | | | | |
| Biryuza | 5.0 | 4.2 | 1.8 | 2.1 | 1.1 |
| Donetski Ugolyok | 5.0 | 3.6 | 1.8 | 2.1 | 0.5 |
| Drogana yellow (к) | 4.8 | 2.6 | 3.1 | 2.8 | 1.3 |
| Amazonka | 4.8 | 2.1 | 2.4 | 0.9 | 0.2 |
| $LSD_{05}$ | *0.8* | *1.1* | *1.3* | *0.7* | *1.0* |

cold. Biennial and perennial fruit-bearing formations were less damaged.

The highest degree of damage (3.1 points) was observed for the Drogana yellow variety, which is considered the standard of frost resistance [Shubenko et al, 2021]. Minimal damage to the fruit-bearing formations was found in the early-ripening variety Zoryana and medium-ripening Alyonushka (0.7–0.9 points).

One-year wood was most severely damaged in the early-ripening Mliivska yellow variety (3.9 points), and the least damages were in the

**Table 2.** The degree of freezing of sweet cherry wood tissues after laboratory experiment (on a 5-point scale), points

| Pomological variety | Tissue | | | | | | | | The sum of points for 1-year-old wood | The sum of points for 2-year-old wood |
|---|---|---|---|---|---|---|---|---|---|---|
| | bark | | cambium | | wood | | core | | | |
| | 1-year-old wood | 2-year-old wood | 1-year-old wood | 2-year-old wood | 1-year-old wood | 2-year-old wood | 1-year-old wood | 2-year-old wood | | |
| Early ripening varieties | | | | | | | | | | |
| Dar Mliyeva | 0 | 1.3 | 4.0 | 2.6 | 2.0 | 1.6 | 2.3 | 3.6 | 8.3 | 9.1 |
| Zoryana (k) | 1.0 | 0 | 4.6 | 0.6 | 0 | 0 | 1.3 | 3.0 | 6.9 | 3.6 |
| Mliivska yellow | 3.3 | 0.3 | 4.6 | 1.6 | 4.0 | 1.0 | 4.0 | 5.0 | 15.9 | 7.9 |
| Medium ripening varieties | | | | | | | | | | |
| Mirage | 0.3 | 0.3 | 2.6 | 0.3 | 0 | 2.0 | 0.6 | 3.6 | 3.5 | 5.9 |
| Alyonushka | 1.0 | 0.6 | 2.6 | 0.6 | 0 | 0.6 | 0.6 | 1.3 | 4.2 | 2.5 |
| Aborygenka | 0.3 | 1.0 | 5.0 | 1.0 | 1.0 | 0.3 | 4.0 | 3.3 | 10.3 | 4.5 |
| Melitopolska krapchasta | 2.0 | 4.6 | 4.6 | 4.6 | 0.3 | 3.0 | 3.0 | 3.6 | 9.9 | 11.5 |
| Meotida (k) | 0 | 0 | 1.6 | 0 | 0 | 1.0 | 0.6 | 1.3 | 2.2 | 2.3 |
| Late ripening varieties | | | | | | | | | | |
| Biryuza | 1.0 | 0 | 5.0 | 0 | 1.3 | 1.6 | 1.3 | 2.6 | 8.6 | 4.5 |
| Donetski Ugolyok | 0.3 | 0.6 | 4.6 | 0.6 | 0.3 | 0 | 3.0 | 3.0 | 8.2 | 3.6 |
| Drogana yellow (к) | 1.6 | 0.6 | 5.0 | 0.6 | 2.6 | 2.3 | 2.0 | 2.3 | 11.2 | 5.2 |
| Amazonka | 0 | 0 | 2.3 | 0 | 0 | 0.3 | 1.6 | 0.6 | 3.9 | 0.9 |
| $LSD_{05}$ | 0.8 | 0.7 | 0.6 | 0.4 | 0.6 | 0.5 | 0.9 | 0.6 | – | – |

medium-ripening Mirage and late-ripening Amazonka (0.8 and 0.9 points, respectively). Two-year-old shoots were damaged up to a maximum of 2.8 points in plants of the Melitopolska krapchasta variety, and least of all in the late-ripening varieties of Amazonka and Donetski Ugolyok.

During the assessment of the freezing degree of one-year-old wood tissues after laboratory freezing, the greatest effect of low temperature was found on the cambial cells of early-ripening varieties, which were damaged almost to the maximum (Table 2).

Bark freezing averaged 1.4 points, and this is the lowest degree of damage among the tissues of one-year wood of early ripening varieties. The most severe bark damage was recorded for the Mliivska yellow variety, and there were no low-temperature damages for the Dar Mliyeva variety. The core was also significantly affected by the cold, where freezing on average for the group of varieties amounted to 2.5 points, with the highest value for the Mliivska yellow variety and the lowest for the Zoryana variety.

The analysis of the results of laboratory freezing showed that the wood of the Zoryana variety was not damaged by low temperature, and the Mliivska yellow tree was affected by 4.0 points, while its total damage in early-ripening varieties was at the level of 2 points.

In the group of medium-ripening varieties, the greatest negative effect of low temperatures was found for the cambium of one-year-old wood, which averaged 3.3 points with the maximum value for the Aborygenka variety and the minimum for the Meotida variety. In addition, damage to the cambium in plants of the Meotida variety was the least among all the studied. Freezing of the cambium was close to the maximum also in plants of the Melitopolska krapchasta variety.

In medium-ripening varieties, after artificial freezing, the wood was practically not damaged. Only for the Aborygenka variety, the degree of its damage was at the level of 1.0 points and 0.3 points for the Melitopolska krapchasta variety.

No threatening effect of low temperature was observed for the state of the bark, where the average degree of damage was only 0.7 points. Compared to the bark, a somewhat higher degree of freezing was noted for the core: 1.7 points on average for the group of medium-ripening varieties. Core damage for the Aborygenka variety was the highest (4 points); a value close to this was recorded for the Melitopolska krapchasta variety.

The core of late-ripening varieties was frozen by an average of 1.9 points, which indicates the insignificant effect of freezing on the overall viability of this tissue. The core tissues were damaged the most by low temperature in plants of the Donetski Ugolyok variety. And in the Biryuza and Amazonka varieties, freezing did not exceed 1.6 points.

A high total degree of tissue damage of one-year-old shoots after freezing at the level of 10.4 points was recorded for the early ripening varieties, and the maximum amount of damage was found for the Mliivska Yellow variety. A lower degree of low-temperature damage was obtained for late ripening cherry varieties (7.9 points on average) with the maximum value for the Aborygenka variety. Varieties of early ripening were minimally damaged (about 6.0 points), with the highest value for the control variety Drogana Yellow.

It is known that two-year-old and perennial wood shows the greatest resistance to low-temperature damage, which in a state of biological rest does not suffer damage when the temperature drops to minus 30 °C [Demirsoy et al, 2022].

The analysis of the state of the two-year-old wood tissues of cherry trees after artificial freezing showed that the early-ripening varieties were damaged to the greatest extent (6.86 points on average), medium-ripening slightly less (5.36) and late-ripening varieties even less (3.55 points) (Fig. 1).

The least damage to the tissues of two-year-old wood was experienced by the Zoryana variety, where there was no damage to the bark and wood, minor damage to the cambium and more noticeable damage to the heartwood.

Analyzing the condition of the tissues of early-ripening varieties after exposure to low temperature, the greatest damage to the core was found, which was 3.86 points on average, and to a lesser extent to the tissues of the cambium, 1.6 points. Minor injury was noted for bark and wood tissues, 0.53 and 0.86 points, respectively.

Total low-temperature damage to the tissues of two-year-old wood of medium-ripe varieties was in the range of 2.3–5.9 points, with the exception of the Melitopolska krapchasta variety, whose injury reached the highest degree among all groups of studied varieties.

With the exception of the Melitopolska krapchasta variety, the bark tissues of medium-ripe varieties were practically not damaged. To an

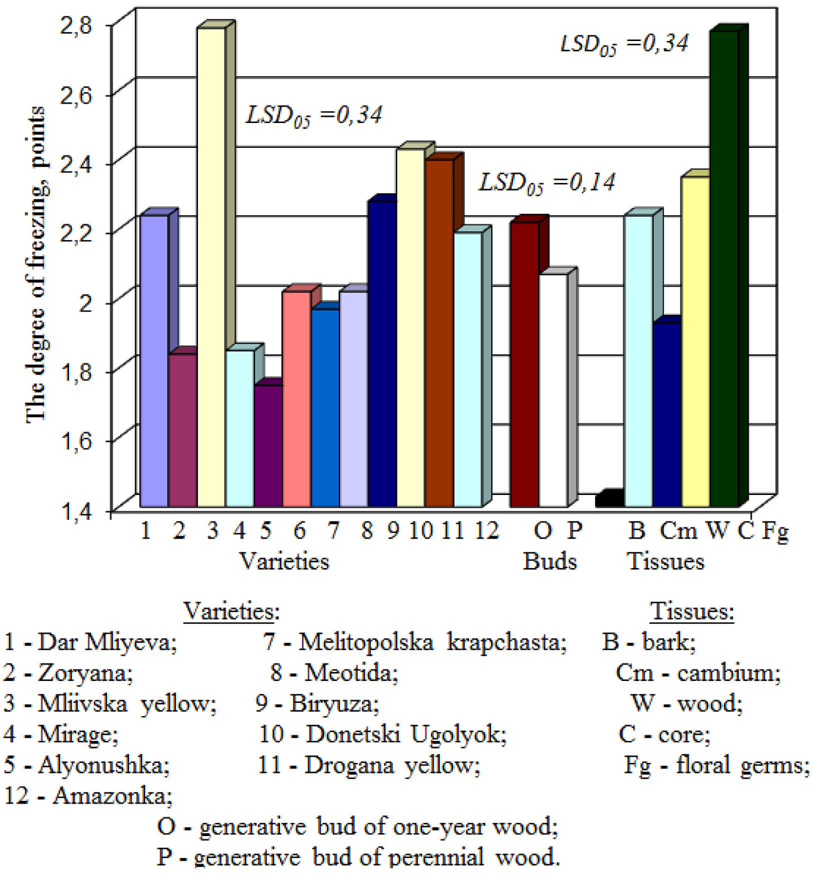


**Figure 1.** The damages to the reproductive and vegetative parts of the cherry shoot

insignificant extent, with the exception of this same variety, the cambium was damaged: a maximum of 1 point for the Aborygenka variety. Wood tissues had a slightly higher degree of damage, especially in trees of the Mirage variety, and the lowest for the Aborygenka variety. In medium-ripe varieties, the core was the least frost-resistant, which was damaged by an average of 2.62 points. Similar to the Melitopolska krapchasta variety, which is the least frost-resistant in this group, the core was also damaged in the Mirage and Aborygenka varieties.

Compared to other studied varieties, the late-ripening varieties were characterized by higher resistance of two-year-old wood tissues to low temperatures. The degree of freezing of tissues on average for the varieties of this group was 3.55 points with the highest value for the control variety Drogana yellow.

The highest degree of tissue freezing was recorded for wood, which averaged 2.13 points for late-ripening varieties. This is especially noticeable for the Donetski Ugolyok variety, where the browning of tissues on the section reached the level of 3 points, and the lowest degree of tissue freezing among all other groups of studied varieties was recorded for the Amazonka variety.

As a result of the artificial freezing of sweet cherry varieties of different ripening periods, it can be concluded that the two-year-old wood of the early-ripening control variety Zoryan, the medium-ripening control Meotida, and the late-ripening Amazonka turned out to be the most frost-resistant.

When determining the frost resistance of different varieties of cherries, special attention was paid to low-temperature damage to the tissues of one-year-old wood, because growth and fruiting processes will depend on this. The obtained data indicate that the cambium and heartwood suffered the greatest low-temperature damage, and the bark and wood suffered much less damage. According to the results of the variance analysis, the dominant influence (42%) on the change in the degree of freezing of one-year branches is caused by the type of tissue. The role of the pomological variety was half as small (23%), and the interaction of the factors was characterized by a much smaller influence (Fig. 2).

As it is known, reproductive buds are most often damaged in drupe crops in winter and spring

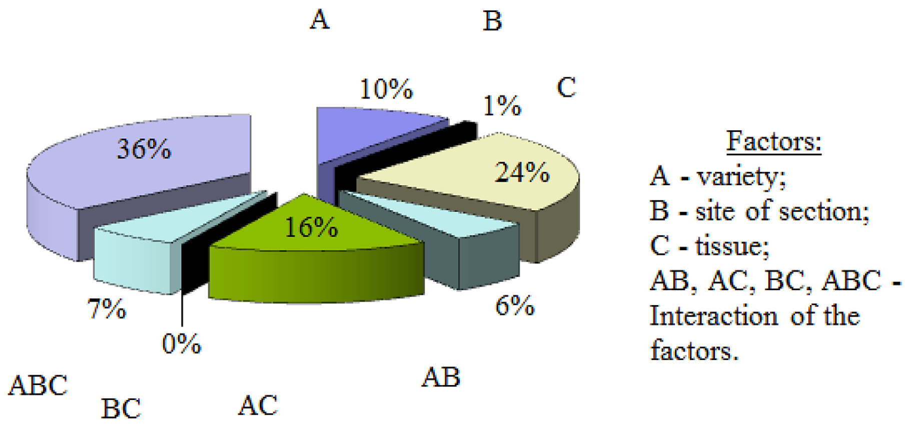


**Figure 2.** Influence of factors on frost resistance of one-year cherry wood (results of multivariate variance analysis)

[Shubenko et al, 2022]. However, this does not significantly affect the general state of the trees, although it can cause mass shedding of ovaries and partial or complete loss of the crop.

As a result of laboratory freezing of branches with annual and perennial fruit-bearing formations, it was found that their frost resistance was almost at the same level and low-temperature damage in general did not exceed 2.2 points.

According to the data obtained by multivariate analysis of variance, the least resistant to the effects of freeze among all studied varieties was the early ripening variety Mliivska yellow (at $LSD_{05}$ = 0.34). Low resistance of fruiting formations was found in the control variety Drogana yellow, which exceeded other varieties in terms of injury level. High resistance to low temperatures was shown by the fruiting formations of the varieties Zoryana (k), Mirage and, especially, Alyonushka. The damage level of other pomological varieties was in the range of 1.9–2.25 points.

A more detailed analysis of damage caused by low temperatures to reproductive buds showed that the most negative effect of low temperatures was caused to flower germs. The average score of their damage was 2.75 points (with $LSD_{05}$ = 0.14), however, damage of this level has little effect on the productivity of trees, since the reproductive bud of trees of drupe species can contain 5-6 or more flower germs [Matzneller et al, 2016].

During artificial freezing, certain negative effects of low temperatures were also experienced by core and cambium tissues, although the level of their damage did not exceed 2.8–2.7 points ($LSD_{05}$ = 0.34). High frost resistance was observed in the bark tissues of fruit formations, where the degree of freezing was 1.42 points.

Analyzing the degree of influence of the studied factors, we again observe the predominant influence of the "tissue" factor, the portion of which is 24%.

## CONCLUSIONS

A significant resistance of the bark to the effect of low temperature was revealed in the group of early-ripening and late-ripening varieties. Compared to other tissues, the average score of its injury was only 0.7 points. The most severe frostbite of the bark was observed for the control variety Drogana yellow, while for the Amazonka variety no damage was noted at all.

Comparing the low-temperature damage to the wood of late-ripening cherry varieties, it should be noted that the highest degree was for the Drogana yellow variety, while no negative impact on the wood was observed for the Amazonka variety.

According to the results of multivariate variance analysis, the highest overall degree of freezing of one-year-old wood after artificial freezing was observed for the varieties Mliivska yellow and Drogana yellow, somewhat less for the varieties Aborygenka and Melitopolska krapchasta. The lowest values of this feature were found for Meotida, Mirage and Amazonka varieties. The obtained experimental data are consistent with the results of the field assessment of winter hardiness, and the pattern of tree damage of the same varieties is preserved.

Judging by the obtained data, the bark tissues of late-ripening varieties were practically not damaged by low temperatures, only for the Biryuza variety their slight darkening was observed.

The cambium of Drogana yellow and Donetski Ugolyok was also slightly damaged. A noticeable negative effect of freezing was found for wood tissues with an average damage of 1.05 points for the group of varieties, and the maximum for the Drogana yellow variety. No negative effect of low temperature was detected for the Donetski Ugolyok variety.

In the group of early ripening, two-year-old wood was frozen the most in trees of the Dar Mliyeva variety, for which the highest damage degree to the tissues of the bark, cambium and wood was observed. Damage to the mentioned tissues was significantly less in plants of the Mliivska yellow variety. However, their cores had the maximum damage not only within the early ripening group, but also among all studied varieties.

With the help of artificial freezing, it was established that the early-ripening control variety Zoryana, medium-ripening control variety Meotida, and late-ripening Amazonka is characterized by the highest frost resistance of annual and perennial wood. The highest resistance of fruiting formations was found in the control early-ripening Zoryana variety, medium-ripening Alyonushka and late-ripening Amazonka variety.

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