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# PROCEEDINGS OF THE 10<sup>TH</sup> INTERNATIONAL HERBAGE SEED CONFERENCE

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# CONTENTS

## KEYNOTE SESSION

|  |    |
|--|----|
| Breeding Improved Cool-season Turfgrass with Improved Seed Production<br>William A. Meyer, Phillip Vines, Stacy A. Bonos, Ronald F. Bara, Dirk A. Smith,<br>and Eric N. Weibel ..... | 3  |
| Herbicide Resistance in Herbage Seed Crops: A Global Perspective<br><i>Carol A. Mallory-Smith</i> .....  | 6  |
| Potential for Quantum Leaps Forward in Agronomic Management of Seed Crops<br><i>Richard J. Chynoweth</i> .....   | 9  |
| Digitalization in Herbage Grass Seed Production and Research: Opportunities and Challenges<br><i>René Gislum, Jacob G. Gyldengren, and Birte Boelt</i> .....                         | 14 |

## ORAL PRESENTATIONS

### Seed Production

|  |    |
|--|----|
| Organic Herbage Seed Production<br><i>Birte Boelt, Henrik Bak Topbjerg, Shuxuan Jing, and René Gislum</i> .....  | 21 |
| Effects of Sowing Methods and Sowing Rates in Organic Seed Production<br><i>Lars T. Havstad and John I. Øverland</i> .....   | 24 |
| The Development of Seed Production of Forage Plantain in New Zealand<br><i>Murray J. Kelly and John D. Foley</i> .....   | 28 |
| Annual Ryegrass Seed Yield Response to Trinexapac-Ethyl: New Zealand and Oregon<br><i>M. Philip Rolston, Nicole P. Anderson, Richard J. Chynoweth, Thomas G. Chastain,<br/>Murray J. Kelly, and Bede L. McCloy</i> ..... | 33 |
| Response of Meadow Bromegrass Seed Crops to Plant Growth Regulators in Canada<br><i>Calvin L. Yoder, Nityananda Khanal, and Talon M. Gauthier</i> .....  | 36 |
| Delivering a Seed Production Course to the World via Online Distance Education<br><i>Alyssa S. DuVal and Thomas G. Chastain</i> .....  | 41 |

### Seed Development and Harvest Efficiency

|  |    |
|--|----|
| Is Red Clover Seed Set Potential Influenced by Pollen Quality?<br><i>Shuxuan Jing, Per Kryger, and Birte Boelt</i> .....   | 51 |
| Harvest Efficiency and Seed Quality in Orchardgrass Seed Production<br><i>Federico Bertuch, Santiago N. Tourn, M. Belén Mermoz, and Pablo Grehan</i> .....   | 55 |
| Seed Yield and Seed Shattering with Different Windrowers in Oregon Grass Seed Crops<br><i>Nicole P. Anderson, Mathis Goussard, Brian C. Donovan, Carol J. Garbacik,<br/>and Thomas G. Chastain</i> ..... | 59 |
| Harvest Timing Determination in Grass Seed Crops by Portable NIR Spectroscopy<br><i>Thomas G. Chastain and Nicole P. Anderson</i> .....  | 63 |

## **Pest Management**

|  |    |
|--|----|
| Soil Tillage Strategy to Reduce Seed Survival of Two Important Annual Grasses<br><i>Peter K. Jensen</i> .....  | 71 |
| Advances in Grass Weed Management in Grasses Grown for Seed in Oregon<br><i>Andrew G. Hulting, Daniel W. Curtis, Kyle C. Roerig, and Carol A. Mallory-Smith</i> .....  | 75 |
| Population Biology and Epidemiology of <i>Claviceps purpurea</i> in Cool-season Grass Seed Crops<br><i>Jeremiah K.S. Dung, Qunkang Cheng, Navneet Kaur, Darrin L. Walenta, Robert A. Cating, Silvia I. Rondon, Kenneth E. Frost, Stephen C. Alderman, and Philip B. Hamm</i> ..... | 78 |
| Discovery and Biological Control Potential of Slug-killing Nematodes in Oregon<br><i>Rory J. McDonnell, Andrew J. Colton, Dana K. Howe, Anh D. Ha, and Dee R. Denver</i> .....   | 85 |

## **Genetics and Breeding**

|   |     |
|---|-----|
| Genetic Variation of Alfalfa Seed Yield in the Establishment Year<br><i>Dura Karagić, Dragan B. Milić, Snežana M. Katanski, Branko R. Milošević, Miroslav Z. Zorić, and Bernadette Julier</i> ..... | 91  |
| Stem Rust Resistance in USDA Perennial Ryegrass Germplasm<br><i>Ryan J. Hayes, Mark D. Azevedo, Lori Evans-Marks, and Dana D. Moore</i> .....   | 95  |
| Kentucky Bluegrass Vernalization Genes<br><i>B. Shaun Bushman, Matthew D. Robbins, Paul G. Johnson, Irina Orlova, and Bob Harriman</i> .....  | 99  |
| Development of New PCR Methods to Distinguish Morphological Similarities in Herbage Seeds<br><i>Yuguo Wu, Dong Luo, Shuheng Shen, Lichao Ma, Xiaowen Hu, Oanrong Wang, and Zhipeng Liu</i> .....    | 101 |

## **POSTER ABSTRACTS**

|  |     |
|--|-----|
| Effects of Sowing Methods and Cover Crops on Tall Fescue Seed Yield<br><i>Serge Bouet, François Deneufbourg, and Guenaëlle Hellou</i> .....  | 107 |
| Topping Effect on White Clover Seed Production in Argentina<br><i>Ezequiel De Bárbara and Martín Borini</i> .....  | 108 |
| Effect of Grazing on Kernza Grain Yield and Lodging in Dual-use Wheatgrass<br><i>Jeremie R. Favre, Jacob M. Jungers, Mitch C. Hunter, Craig C. Sheaffer, and Valentin D. Picasso</i> ..... | 109 |
| Plant Growth Regulator Combination Effects on Tall Fescue Seed Crops<br><i>Tara M. Hudgins, Nicole P. Anderson, Thomas G. Chastain, and Carol J. Garbacik</i> .....                        | 110 |
| Seed Crop Agronomy of Creeping Red Fescue in Canada's Peace Region<br><i>Nityananda Khanal, Calvin L. Yoder, and Talon M. Gauthier</i> .....   | 111 |
| Row Spacing Influences Seed Yield and Yield Components in Alfalfa Seed Production<br><i>Peisheng Mao, Hui Wang, Wenxu Zhang, Mingya Wang, and Chengming Ou</i> .....                       | 112 |
| Accumulation and Partitioning of Nutrients by Hybrid Carrot Grown for Seed<br><i>Amber D. Moore, Ekaterina A. Jeliakova, and Tracy M. Wilson</i> .....                                     | 113 |

|   |     |
|---|-----|
| Trinexapac-ethyl Timing and Rate Effects on Crimson Clover Seed Production<br><i>Mohammed M. Morad, Nicole P. Anderson, and Thomas G. Chastain</i> .....  | 114 |
| Seed Production of Endophyte-free and Infected Tall Fescue Under Two Defoliation Frequencies<br><i>Lucas R. Petigrosso, Diego Navarro, Silvia G. Assuero, Osvaldo R. Vignolio,<br/>Jorge A. Castaño, and Mabel N. Colabelli</i> ..... | 115 |
| Effects of Plant Growth Regulators and Nitrogen on Kentucky Bluegrass Seed Production<br><i>Ruijun Qin, Nicole P. Anderson, Darrin L. Walenta, and Scott B. Lukas</i> .....   | 116 |
| <i>Panicum coloratum</i> Seed Yield Components in a Farmer’s Field Under Diverse Fertilization<br><i>Juan Mattera, María A. Tomás, Mauro Lifschitz, Mabel Giordano, and Ezequiel Pacente</i> .....                                    | 117 |
| Perception of Factors Affecting Tropical Forage Grass Seed Production in Argentina<br><i>María A. Tomás and Juan Mattera</i> .....  | 118 |
| First Detection of <i>Coleophora deauratella</i> in Northeastern Oregon Red Clover Seed Production<br><i>Darrin L. Walenta and Nicole P. Anderson</i> .....   | 119 |
| Perennial Ryegrass Response to Trinexapac-ethyl Products, Rates, and Timing<br><i>Johan Wander</i> .....  | 120 |
| Effect of Combine Speed on White Clover Harvest Efficiency<br><i>Federico Bertuch, Ezequiel De Bárbara, and Martín Borini</i> .....   | 121 |
| Perennial Ryegrass Seed Loss at Harvest<br><i>Richard J. Chynoweth and M. Phil Rolston</i> .....  | 122 |
| Seed Processing Methods for Improved Sowing in <i>Bromus auleticus</i> Trinius<br><i>Lucas D. Garro, Rosa N. Jauregui, Sabrina Miri, Guillermo Azcarate, Diego Andregnette,<br/>and Juan Amadeo</i> .....                             | 123 |
| Effect of Cultivars and Environment on Seed Quality in <i>Vicia sativa</i><br><i>Xiaowen Hu, Lingjie Yang, Dali Chen, and Yanrong Wang</i> .....  | 124 |
| Effect of Seed Coat Semipermeable Layer on Electrical Conductivity Seed Vigor Assessment<br><i>Yanrong Wang, Yanyan Lv, Xueqing He, and Xiaowen Hu</i> .....  | 125 |
| Effect of <sup>12</sup> C <sup>6+</sup> Heavy Ion Irradiation on Germination of <i>Vicia unijuga</i> seeds<br><i>Jie Li, Tiantian Ma, Zhuanzhuan Yan, Qi Yan, Bin Nie, and Jiyu Zhang</i> .....                                       | 126 |
| Untangling Weed Problems and Crop Rotation Options in Grass Seed Production<br><i>George W. Mueller-Warrant</i> .....   | 127 |
| Green Leaf Volatiles Prime for Wound Response in Grasses<br><i>James E. Dombrowski, Vicky G. Hollenbeck, Brent Kronmiller, and Ruth C. Martin</i> .....   | 128 |
| A Method to Select for Root Chelation Activity in Annual Ryegrass<br><i>Mark D. Azevedo and Ryan J. Hayes</i> .....   | 129 |
| Genetic Diversity of <i>Puccinia graminis</i> f. sp. <i>lolii</i> from the Willamette Valley<br><i>Dana D. Moore, Mark D. Azevedo, and Ryan J. Hayes</i> .....  | 130 |
| Development of 3-D Topometric Imaging for High-throughput Phenotyping in Perennial Ryegrass<br><i>Travis B. Tubbs and Thomas G. Chastain</i> .....  | 131 |

|   |     |
|---|-----|
| Susceptibility of Grass Cultivars to Barley Yellow Dwarf Viruses<br><i>Ruth C. Martin, Léa Merlet, Thomas C. Lockwood, and James E. Dombrowski</i> .....  | 132 |
| Choke Spread and Evaluation of Orchardgrass Germplasm for Choke Resistance<br><i>Léa Merlet, Ruth C. Martin, B. Shaun Bushman, Thomas C. Lockwood, Patrick Boren, and James E. Dombrowski</i> .....                     | 133 |
| Identification of <i>HSP70</i> Family Stress Response Genes in <i>Cleistogenes songorica</i><br><i>Yufei Zhang, Zhuanzhuan Yan, Tiantian Ma, Qi Yan, Fan Wu, Xueyang Min, Jie Li, Yufeng Zhao, and Jiyu Zhang</i> ..... | 134 |

## GENETIC VARIATION OF ALFALFA SEED YIELD IN THE ESTABLISHMENT YEAR

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### Abstract

In Serbia, alfalfa (*Medicago sativa* L.) is grown on about 140,000 ha, out of which 3–7% are intended for seed production. Climatic factors (amount and distribution of precipitation) are the main determinants of alfalfa seed yield. Average alfalfa seed yield in Serbia is about 250 kg ha<sup>-1</sup>, and seed production is characterized by huge variation (15–800 kg ha<sup>-1</sup>). The most challenging year for seed production is the establishment year, i.e., the first year of plant life. To be successful on the market, new alfalfa varieties are selected for high forage production, quality of forage, and persistence. However, outstanding seed-yield potential is also needed. Even if breeding for seed yield is not prioritized, genetic variation among cultivars is known. In order to explore genetic variation for alfalfa seed yield and its components in the establishment year, a trial with 400 accessions was established at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia, on May 21, 2018. The genotypes were tested in a partially replicated design. For the purpose of this study, we extracted model best linear unbiased predictions (BLUPs) for 20 populations, which represent a part of the European alfalfa core collection. The plot size was 6 m<sup>2</sup>. Seed yield and its components were recorded for each plot. The 2018 field season was not favorable for alfalfa seed production in Serbia, but the results clearly demonstrate differences among varieties for total seed yield and its components in the establishment year. Seed yields varied from 31.8 kg ha<sup>-1</sup> for the variety ‘Tereza’ to 96.7 kg ha<sup>-1</sup> for the variety ‘Etincelle’. Higher seed yields were obtained with less dormant varieties (dormancy ratings 5–6), while lower yields were recorded with more dormant varieties (dormancy ratings 3–4). Analyses showed that, even under unfavorable conditions, genetic variation could be important for alfalfa seed production in the year of establishment.

**Keywords:** alfalfa, seed yield, variety, genetic variation

### Introduction

In Serbia, alfalfa (*Medicago sativa* L.) is grown on about 140,000 ha, out of which 3–7% are intended for seed production. Climatic factors (amount and distribution of precipitation) are the main determinants of alfalfa seed yield. Average alfalfa seed yield in Serbia is about 250 kg ha<sup>-1</sup>, and seed production is characterized by a huge variation (15–800 kg ha<sup>-1</sup>). The most challenging year for seed production is the establishment year, i.e., the first year of plant life. To be successful on the market, new alfalfa varieties are selected for high forage production, high quality of forage, and persistence. However, outstanding seed-yield potential is also needed. Even if breeding for seed yield is not prioritized, genetic variation among cultivars is known.

The main goals of the research were to: (1) evaluate seed yield and its components in one part of the European alfalfa core collection in the year of establishment, (2) analyze phenotypic correlations among

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the main seed yield traits, and (3) compare distribution of the examined traits in total genetic variation. The probability of exploring genetic variation in breeding programs is considered.

## Methods

In order to explore genetic variation for alfalfa seed yield and seed-yield components in the establishment year, a trial with 400 accessions was established at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia on May 21, 2018. The experimental site was located in northern Serbia, at 45°20' N, 19°51' E, at 80 m above sea level. This area has a continental semiarid to semihumid climate, a mean monthly air temperature of 11°C, annual total precipitation of around 600 mm, and a highly uneven distribution of precipitation. The genotypes were tested in a partially replicated design. The design grid size was 10 rows by 44 columns. The proportion of replicated genotypes was 0.14. The data on the measured traits were analyzed by the ASReml-R package mixed model approach (Butler et al., 2009).

Furthermore, the subsets of best linear unbiased predictions (BLUPs) from the model were used for visualizations by means of simple scatter plot graph and multivariate principal component analysis (PCA) technique. The biplot graph was used as visual display of the genotype-by-trait relationship.

For the purpose of this study, we extracted model BLUPs for 20 populations, which represent a part of the European alfalfa core collection: 11 from France ('Artemis', 'Dorine', 'Etincelle', 'Flamande', 'Galaxie', 'Lusiente', 'Luzelle', 'Mezzo', 'Milky Max', 'Sanditi', 'Timbale'), 4 from Serbia ('Banat VS', 'K22', 'K28', 'Nijagara'), 3 from the Czech Republic ('Holyna', 'Niva', 'Tereza'), 1 from Sweden ('SW Nexus'), and 1 from Denmark ('Creno'), with dormancy ratings of 3 to 6. The plot size was 6 m<sup>2</sup>. Seed yield (SY, kg ha<sup>-1</sup>) and its components (plant height in full flowering, (PHF, cm), stems m<sup>-2</sup> (SM), fertile stems m<sup>-2</sup> (FSM), number of inflorescences per stem (IS), number of pods per inflorescence (PI), seeds per pod (SP), and 1,000-seed weight (TSW)) were recorded for each plot.

## Results and Discussion

The results clearly demonstrate the influence of weather conditions on alfalfa SY and its components in the establishment year. Average SY in the trial was 64.4 kg ha<sup>-1</sup>. The highest seed yield was recorded with variety 'Etincelle' (96.7 kg ha<sup>-1</sup>), while the lowest yields were found in variety 'Tereza' (31.8 kg ha<sup>-1</sup>). A huge SY variation was recorded in the trial, from 7.8 kg ha<sup>-1</sup> within varieties 'Lusiente' and 'Tereza' to 140.2 kg ha<sup>-1</sup> within the variety 'Sanditi'. There is a strong correlation between alfalfa seed yield and unfavorable agroecological conditions. The average number of SP was 2.6, while the mean value for PI was 7.6. The average number of FSM was 254.7. Mean PHF was 79.3 cm, ranging from 69 cm to 93 cm.

The highest positive phenotypic correlations were found between SY and PI, FSM, and SP (Table 1) in the year of establishment. Also, the highest positive correlations in the trial were found between SM and FSM (0.78), as expected. Similar phenotypic correlations between seed yield and seed yield components were reported by Karagić (2004), with one main difference regarding climatic conditions; Karagić's observations were reported for the year 2000, which was favorable for alfalfa seed yields in Serbia in terms of weather conditions.

Figure 1 depicts the relationship between SY and PI. These two traits are the most important for successful alfalfa seed production. Bolaños Aguilar et al. (2000) reported strong high positive correlations between these two traits in alfalfa. Figure 1 clearly shows that populations were divided into four groups. The upper right quadrant contains the accessions that have high SY and a high PI ('Etincelle', 'Mezzo',

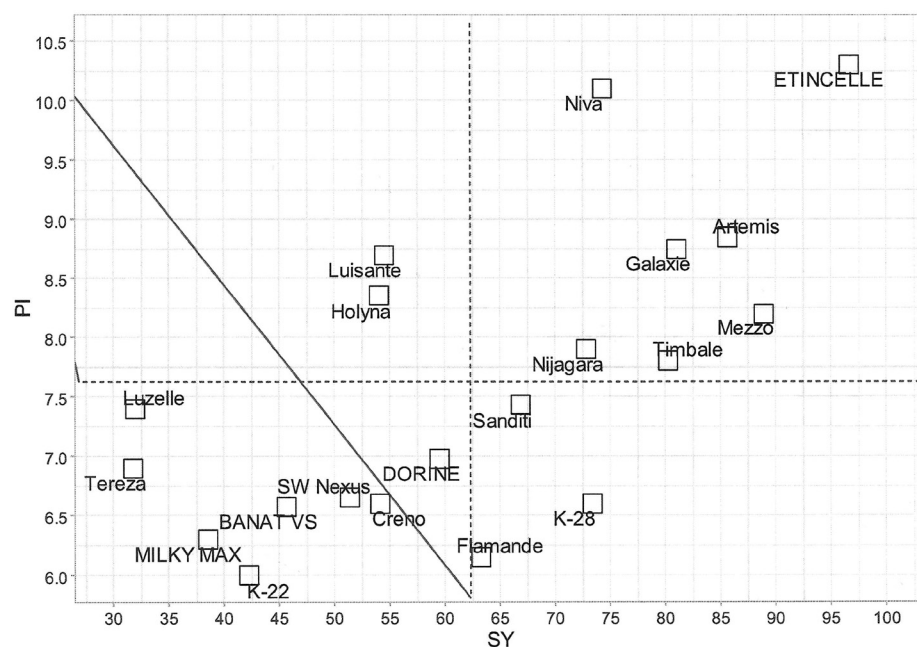


**Table 1.** Phenotypic correlations (Pearson coefficient) between seed yield and seed yield components.<sup>1,2</sup>

|     | PHF    | SY      | SM       | FSM    | IS      | PI     | SP    | TSW   |
|-----|--------|---------|----------|--------|---------|--------|-------|-------|
| PHF | 1.000  |         |          |        |         |        |       |       |
| SY  | -0.055 | 1.000   |          |        |         |        |       |       |
| SM  | -0.115 | 0.491*  | 1.000    |        |         |        |       |       |
| FSM | -0.054 | 0.589** | 0.781*** | 1.000  |         |        |       |       |
| IS  | -0.137 | 0.406   | 0.155    | 0.253  | 1.000   |        |       |       |
| PI  | 0.382  | 0.649** | 0.249    | 0.441  | 0.522*  | 1.000  |       |       |
| SP  | -0.128 | 0.575** | 0.270    | 0.457* | 0.599** | 0.516* | 1.000 |       |
| TSW | -0.108 | 0.205   | 0.116    | 0.154  | 0.228   | -0.167 | 0.218 | 1.000 |

<sup>1</sup> PHF = plant height in full flowering; SY = seed yield; SM = stems m<sup>-2</sup>; FSM = fertile stems m<sup>-2</sup>; IS = inflorescences per stem; PI = pods per inflorescence; SP = seeds per pod; TSW = thousand-seed weight

<sup>2</sup>Significance levels: \*0.05; \*\*0.01; \*\*\*0.001

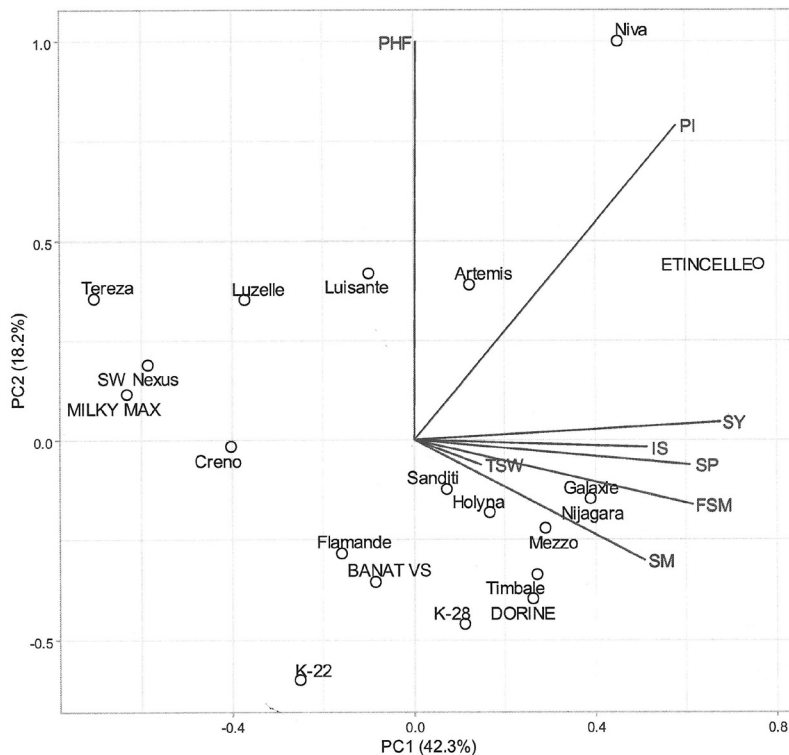


**Figure 1.** Relationship between seed yield (SY) and number of pods per inflorescence (PI) of the examined alfalfa varieties.

‘Artemis’, ‘Timbale’, ‘Galaxie’, ‘Niva’, and ‘Nijagara’). The lower left quadrant includes genotypes with the lowest SY and PI in the year of establishment.

The PCA biplot in Figure 2 shows the relationship between genotypes and SY and its components. The biplot explains about 60% of the total phenotypic variation. Most of the populations are concentrated on the right side of the first dimension. These genotypes (‘Galaxie’, ‘Nijagara’, ‘Mezzo’, ‘Holyňa’, ‘Sanditi’) were characterized by high values of highly correlated traits (SY, IS, SP, FSM, SM, and TSW), based on the biplot pattern. On the negative side of the first dimension are the genotypes ‘Tereza’, ‘SW Nexus’, ‘Milky Max’, and ‘Creno’, which present more dormant varieties (dormancy ratings 3–4) in the trial. Moreover, the biplot shows overall negative correlations among PHF and the groups of highly correlated traits. The lack of relationship between PHF and other traits is due to the unfavorable weather conditions during the trial, as well as the relation between plant height and lodging in alfalfa.





**Figure 2.** Biplot principal component analysis (PCA) showing the interrelationship between 20 alfalfa populations and the examined traits.

## Conclusion

The relationships between SY and SY components presented in this paper are in line with previous research related to alfalfa seed yield performance. Breeding objectives should take into account alfalfa SY *per se*, but also PI and SP. To an extent, genetic variation in alfalfa is likely to be very dependent on climatic conditions in a particular year.

Higher SY was obtained from the less dormant varieties (dormancy ratings 5–6), while lower yields were recorded in the more dormant varieties (dormancy ratings 3–4). Overall, the analyses showed that, even under unfavorable conditions, genetic variation could be important for alfalfa seed production in the year of establishment. Further analyses are needed to reach a final conclusion and carry out tandem selection for both forage yield traits and seed yield.

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