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FOREWORD

A Word from the Editor-in-Chief

Dear colleagues,

In your hands is the Book of Proceedings of the X International Scientific Agricultural Symposium “AGROSYM 2019”, which I hope you will find useful in your work. As many as 900 contributions, from 82 countries, have been accepted for oral or poster presentations. Symposium themes cover all branches of agriculture and are divided into 7 sessions: 1) Plant production, 2) Plant protection and food safety, 3) Organic agriculture, 4) Environmental protection and natural resources management, 5) Animal husbandry, 6) Rural development and agro-economy, 7) Forestry and agroforestry. Papers dealing with agricultural engineering and technology were included into one of the seven sessions depending on their focus.

In the plenary lectures were addressed interesting topics; one keynote was on biotechnology and two others dealt with organic farming in Australia and Europe. This confirms the role of AGROSYM as a forum for open discussions and exchanges on agriculture, food, the environment and rural development in the Balkans and beyond. Many of the papers identify a number of approaches and market-based incentives to encourage producers to achieve higher levels of performance (from both economic and environmental points of view) and as a result to meet the expectations of governments and consumers.

The successful management of agricultural resources to satisfy changing human needs, while maintaining or enhancing the quality of the environment and conserving natural resources, indicate a long-term agricultural development imperative. Advances in productivity, profitability and stability of modern cropping, animal and forestry systems will have to be achieved globally on an ecologically sustainable basis. Today, it is obvious that conventional methods of agricultural production, while providing sufficient food and various products to humanity, have led to a number of negative impacts, including the transgression of many planetary boundaries. These negative impacts raise serious questions about the long-term sustainability of high-input agriculture and call for a genuine transition towards sustainable agro-food systems, which achieve food and nutrition security for present and future generations within the safe operating space for humanity.

Full texts of the submitted communications will be available on the website of AGROSYM (<http://agrosym.ues.rs.ba>). Each paper included in the present Book of Proceedings was positively reviewed.

Much appreciation is due to the authors of all papers submitted and presented at the symposium as well as to all symposium participants whose ideas and contributions allowed rich and lively discussions during the various sessions. Many thanks to all reviewers, session moderators and colleagues for their help in editing the Book of Proceedings. Special thanks go to all co-organizers, partners and sponsors for their unselfish collaboration and comprehensive support.

Editor-in-Chief



Dusan Kovacevic, PhD

East Sarajevo, 12 October 2019

CONTENT

1. PLANT PRODUCTION	34
NUTRIENTS STATUS IN MAIZE GRAIN FROM SUSTAINABLE AGRICULTURE	
Vesna DRAGIČEVIĆ, Milena SIMIĆ, Milan BRANKOV, Milovan STOILJKOVIĆ, Branka KRESOVIĆ, Miodrag TOLIMIR.....	35
INCREASE IN GROWTH AND YIELD OF SOYBEAN IN FIELD TRIAL BY IAA-PRODUCING MUTANT <i>BACILLUS</i> SP. CO-INOCULATED WITH <i>B. JAPONICUM</i>	
Renata ILIČIĆ, Dragana LATKOVIĆ, Dragana JOŠIĆ	41
EVALUATION OF RAPE SEED GENETIC DIVERSITY AND CREATION OF DROUGHT-RESISTANT GENOTYPES USING <i>IN VITRO</i> CELL CULTURE	
Oksana KLYACHENKO, Larysa PRYSIAZHNIUK, Tetiana DUDKA.....	47
CULTIVATION PRACTICES EFFECT <i>CANNABIS SATIVA</i> YIELD	
Eleni WOGIATZI-KAMWOUKOU, Kyriakos D. GIANNOULIS, Eleftheria Anna PORICHI, Nikolaos GOUGOULIAS, Dimitrios KALFOUNTZOS, Dimitrios BILALIS	56
TEMPERATURE EFFECT ON SEED GERMINATION RATES OF DIFFERENT WINTER LEGUMES AND SPRING CEREALS	
Elpiniki SKOUFOGIANNI, Kyriakos D. GIANNOULIS, Dimitrios BARTZIALIS, Foteini LAVDI, Nicholas G. DANALATOS.....	64
RESPONSE OF SUNFLOWER HYBRIDS TO STAND DENSITY	
Igor BALALIĆ, Jovan CRNOBARAC, Vladimir MIKLIČ, Velimir RADIĆ, Nenad DUŠANIĆ	70
INFLUENCE OF AGROTECHNICAL MEASURES ON YIELD AND QUALITY OF GREEN ONION	
Aleksandra GOVEDARICA-LUČIĆ, Goran PERKOVIĆ, Alma RAHIMIĆ, Jelena PLAKALOVIĆ.....	75
RESPONSE OF SOME WHEAT GENOTYPES TO DROUGHT AT GERMINATION AND EARLY SEEDLING GROWTH	
Mirjana JOVOVIĆ, Vesna TUNGUZ, Milan MIROSAVLJEVIĆ, Novo PRŽULJ, Zoranka MALEŠEVIĆ.....	79
SOWING QUALITIES AND YIELDING PROPERTIES OF SUDAN GRASS (<i>Sorghum sudanense</i>) DEPENDING ON FRACTIONATION OF SEEDS AND SEEDING RATE	
Tatiana KRIUKOVA, Georgiy SHENTSEV	86
IDENTIFICATION OF MOLECULAR MARKERS FOR FOREGROUND AND BACKGROUND SELECTION IN <i>Ga1-S</i> INCORPORATION INTO MAIZE LINES	
Marija KOSTADINOVIĆ, Dragana IGNJATOVIĆ-MIČIĆ, Jelena VANČETOVIĆ, Danijela RISTIĆ, Ana OBRADOVIĆ, Olivera ĐORĐEVIĆ MELNIK, Anika KOVINČIĆ	91
EFFECT OF CULTIVAR ON THE YIELD AND ANTIOXIDANT ACTIVITY OF OKRA (<i>Abelmoschus esculentus</i> L. Moench) GROWN IN SLOVAK REPUBLIC	
Miroslav ŠLOSÁR, Ondrej HEGEDŰS, Ivana MEZEYOVÁ, Alžbeta HEGEDŰSOVÁ, Ján FARKAŠ	96

RESULTS OF TESTING THE SOWING AGGREGATES FOR PLANTING THE NARROW-ROW CROPS WITH CONVENTIONAL AND REDUCED TILLAGE	
Saša BARAĆ, Milan BIBERDŽIĆ, Dragan PETROVIĆ, Aleksandar ĐIKIĆ, Rade RADOJEVIĆ, Aleksandar VUKOVIĆ, Dragoslav ĐOKIĆ	102
THE SIGNIFICANCE OF CLIMATE VARIABILITY ON THE PRODUCTION OF WHEAT AND RAPESEED IN SERBIA	
Jasmina KNEŽEVIĆ, Miroljub AKSIĆ, Dalibor TOMIĆ, Vera DJEKIĆ, Nebojša GUDŽIĆ, Dragan TERZIĆ, Dobrivoj POŠTIĆ, Rade STANISAVLJEVIĆ	108
EFFECT OF CULTIVAR AND YEAR ON YIELD AND GRAIN QUALITY OF TWO-ROW SPRING BARLEY	
Milomirka MADIĆ, Dragan ĐUROVIĆ, Aleksandar PAUNOVIĆ, Vladeta STEVOVIĆ, Dalibor TOMIĆ, Desimir KNEŽEVIĆ, Vera ĐEKIĆ	113
CROP ROTATION PRODUCTIVITY WITH CEREALS AND LEGUMES: A SHORT REVIEW	
Elpiniki SKOUFOGIANNI, Kyriakos D. GIANNOULIS, Dimitrios BARTZIALIS, Georgios CHARVALAS, Nicholaos G. DANALATOS	119
INTERCROPPING – MAY BE AN OLD PRACTICE BUT TIMELESS: A SHORT REVIEW	
Elpiniki SKOUFOGIANNI, Kyriakos D. GIANNOULIS, Dimitrios BARTZIALIS, Georgios CHARVALAS, Nicholaos G. DANALATOS	126
A MISUNDERSTANDING BUT VERY PROMISING CROP: LUPINS	
Elpiniki SKOUFOGIANNI, Kyriakos D. GIANNOULIS, Dimitrios BARTZIALIS, Georgios CHARVALAS, Foteini LAVDI, Nicholaos G. DANALATOS	133
ASSESSMENT OF CEREAL SPECIES BASED ON YIELD AND AGRO-PHYSIOLOGICAL PARAMETERS UNDER RAINFED CONDITION	
İrfan ÖZTÜRK	139
EFFECT OF YEAR AND FERTILIZATION ON WINTER BARLEY QUALITY	
Vera RAJIĆIĆ, Jelena MILIVOJEVIĆ, Dragan TERZIĆ, Milan BIBERDŽIĆ, Ranko KOPRIVICA, Dragan GRČAK, Milosav GRČAK.....	146
GRAIN YIELD AND QUALITY OF WINTER WHEAT CULTIVARS	
Vera RAJIĆIĆ, Vesna PERIŠIĆ, Milomirka MADIĆ, Vera POPOVIĆ, Vladimir PERIŠIĆ, Kristina LUKOVIĆ, Dragan TERZIĆ.....	152
PRODUCTIVITY AND BIOCHEMICAL COMPOSITION OF SOYBEAN GRAIN, DEPENDING ON THE MEANS OF PROTECTION	
Tatyana ASEEVA, Anna TISHKOVA, Natalia SAVCHENKO	158
FORMATION OF SPRING TRITICALE GRAIN YIELD IN CONDITIONS OF MONSOON CLIMATE	
Tatyana ASEEVA, Kristina ZENKINA, Egor TOLOCHKO	162
FEATURES OF THE INFLUENCE OF CLIMATIC FACTORS ON THE CROP AND QUALITY OF GRAIN OF SPRING OATS	
Tatyana ASEEVA, Irina TRIFUNTOVA, Egor TOLOCHKO	167
GENOTYPE BY ENVIRONMENT INTERACTIONS ON NINE COTTON FIBER TRAITS	
Vasileios GREVENIOTIS, Evangelia SIOKI, Constantinos G. IPSILANDIS	172
ASSESSING YIELD OF WINTER CEREALS UNDER EXTREME CONDITIONS IN GREECE	

Vasileios GREVENIOTIS, Stylianos ZOTIS, Evangelia SIOKI, Constantinos G. IPSILANDIS	176
THE INFLUENCE OF AGRO-ECOLOGICAL CONDITIONS ON THE QUALITY OF FIELD PEA	
Igor ĐURĐIĆ Vesna MILIĆ, Branka GOVEDARICA, Saša LALIĆ, Željko LAKIĆ .180	
EFFECT OF THE 1BL.1RS WHEAT-RYE CHROMOSOMAL TRANSLOCATION IN BREAD WHEAT CULTIVARS ON PHYSIOLOGICAL TRAITS	
Fokion PAPATHANASIOU, Ioannis TASIOS, Dimitrios TRAKOSIARHS, Fani KOYTZOYRA, Athanasios G. MAVROMATIS, Chryssanthi I. PANKOU, Ioannis N. XYNIAS.....	186
COMPARISON OF SPECIES COMPOSITION OF VASCULAR PLANTS IN AGROFORESTRY SYSTEM	
Helena HANUSOVA, Jan WINKLER, Lucie CHMELIKOVA, Nicole MAIER, Kurt-Jürgen HÜLSBERGEN.....	191
THE EFFECT OF DROUGHT STRESS ON FRUIT AND SEED COLOR IN PUMPKIN (<i>Cucurbita pepo</i> L.) GENOTYPES	
Musa SEYMEN, Önder TÜRKMEN, Duran YAVUZ, Atilla DURSUN, Ertan Sait KURTAR	197
THE COMPOSITION OF POLLENIZERS FOR SWEET CHERRY (<i>Prunus avium</i> L.) CULTIVARS RELEASED IN THE REPUBLIC OF SERBIA	
Sanja RADIČEVIĆ, Slađana MARIĆ, Milica FOTIRIĆ-AKŠIĆ, Radosav CEROVIĆ, Milena ĐORĐEVIĆ, Nebojša MILOŠEVIĆ, Ivana GLIŠIĆ	205
MOLECULAR CHARACTERISATION OF MAIZE HYBRIDS	
Danijela RISTIĆ, Marija KOSTADINOVIĆ, Natalija KRAVIĆ, Anika KOVINČIĆ, Ana OBRADOVIĆ, Milan STEVANOVIĆ, Jovan PAVLOV	211
THE UTILITY OF AGRO-MORPHOLOGICAL DESCRIPTORS IN UNIFORMITY AND STABILITY DETERMINATION OF MAIZE INBREDS	
Anika KOVINČIĆ, Ksenija MARKOVIĆ, Danijela RISTIĆ, Vojka BABIĆ, Marija KOSTADINOVIĆ, Natalija KRAVIĆ	216
THE INFLUENCE OF CONTINUOUS ANTHROPOGENIC PRESSURE ON FERTILITY AND BIOLOGICAL ACTIVITY OF SOILS	
Tatyana ASEEVA, Natalia SAVCHENKO, Natalia SELEZNEVA, Sergey SHMIGIRILOV	223
QUANTITATIVE PHENOTYPIC CHARACTERISTICS OF PARENT PLANTS OF SOYBEAN CULTIVAR BATYA	
Tatyana ASEEVA, Tamara FEDOROVA, Sergey SHUKYUROV, Natalia SAVCHENKO	230
THE FIRST RECORD OF <i>RATTUS NORVEGICUS</i> ON THE ISLAND OF CYPRUS AND THE EXPECTED EFFECT ON AGRICULTURE AND LOCAL BIOTA	
Eleftherios HADJISTERKOTIS, George KONSTANTINOU, Daria SANNA, Monica PIRASTRU, Paolo MEREU	236
INFLUENCE OF INTERCROPPING MAIZE WITH COWPEA AND FERTILIZATION WITH CLINOPTILOLITE ON FORAGE YIELD AND QUALITY	
Ivan HORVATIĆ, Martina KOVAČEVIĆ, Dubravko MAČEŠIĆ, Darko UHER	242

UPTAKE OF PHOSPHORUS AND POTASSIUM IN SORGHUM PLANTS IN DEPENDENCE ON NUTRITIONAL LEVEL	
Ivan VELINOV	248
STUDIES ON STABILITY OF GRAINS WEIGHT FROM PANICLE TO A COLLECTION OF OATS AUTUMN (<i>Avena Sativa</i> L.) GENOTYPES	
Emilian MADOSA, Giancarla VELICEVICI, Sorin CIULCA, Adriana CIULCA, Constantin AVADANEI, Lavinia SASU, Marcel DANCI	254
GENETIC ANALYSIS OF CHLOROPHYLL CONTENT IN BARLEY (<i>HORDEUM VULGARE</i> L.)	
Emilian MADOSA, Giancarla VELICEVICI, Adriana CIULCA, Sorin CIULCA	261
BREEDING VEGETABLE ROOTSTOCKS TOWARD COPING WITH BIOTIC AND ABIOTIC STRESSES	
Rezq BASHEER-SALIMIA, Doaa NAJAJREH	267
EFFECT OF DIFFERENT DOSES OF NITROGEN FERTILIZER AND VARIETY ON THE YIELD AND GRAIN QUALITY OF WINTER TRITICALE	
Dragana LALEVIĆ, Milan BIBERDŽIĆ, Lidija MILENKOVIĆ	270
CHANGE IN ACIDITY AND MOBILE ALUMINIUM LEVELS IN FOREST, MEADOW AND ARABLE LAND PSEUDOGLEY SOILS	
Marijana DUGALIĆ, Ljiljana BOŠKOVIĆ-RAKOČEVIĆ, Goran DUGALIĆ	276
THE USE OF WASTEWATER FROM THE RECIRCULATING AQUACULTURE SYSTEM FOR BASIL CULTIVATION AND ITS EFFECTS ON THE ESSENTIAL OIL COMPOSITION	
Marian BURDUCEA, Ivayla DINCHEVA, Ilian BADJAKOV, Lenuta DIRVARIU, Alin-Cristian BARBACARIU, Valtcho D. ZHELJAZKOV	282
COMPARISON OF COMMON FERTILIZERS AND FERTILIZERS WITH NITRIFICATION INHIBITOR TO YIELD AND PROTEIN CONTENT ON TRITICUM DURUM	
Kyriakos D. GIANNOULIS, Eleni WOGIATZI-KAMWOUKOU, Evangelos KARAKASIS, Anastasios KARAKASIS, Ilias PLATIS	288
VARIABILITY OF LENGTH OF SPIKE AND NUMBER OF SPIKELETS PER SPIKE IN WHEAT (<i>Triticum aestivum</i> L.)	
Desimir KNEZEVIC, Danica MICANOVIC, Veselinka ZECEVIC, Gordana BRANKOVIC, Danijela KONDIC, Adriana RADOSAVAC, Mirela MATKOVIC STOJSIN, Sretenka SRDIC, Srdjan ATANASIJEVIC, Dusan UROSEVIC	295
PISTACHIO KERNEL CONTENT OF MINERAL ELEMENTS, PROTEIN AND TOTAL OIL UNDER RAIN-FED CULTIVATION IN SYRIA	
Najwa M. ALHAJJAR, Talat AMER, Bayan M. MUZHER	300
GENOTYPE ENVIRONMENT (GXE) INTERACTION AND ASSESSMENT OF BREAD WHEAT (<i>Triticum aestivum</i> L.) GENOTYPES UNDER RAINFED CONDITION	
İrfan ÖZTÜRK	306
INVESTIGATION OF THE EFFECT OF CALCIUM APPLICATIONS ON PLANT DEVELOPMENT OF PEPPER PLANT IN SALT STRESS	
Fikret YASAR, Ozlem UZAL	312

INFLUENCE OF TWO APPLE ROOTSTOCKS ON LEAVES AND FRUITS MINERAL CONTENT OF STARKRIMSON CULTIVAR UNDER RAINFED CONDITIONS	
Bayan M. MUZHER, Ola T. ALHALABI, Talaat AMER, Sami HENNAWI.....	318
EFFECT OF DIFFERENT SEED TREATMENTS ON DORMANCY BREAKING AND GERMINATION OF ACER CAPILLIPES MAXIM.	
Marija MARKOVIĆ, Mihailo GRBIĆ, Dragana SKOČAJIĆ, Matilda ĐUKIĆ, Danijela ĐUNISIJEVIĆ-BOJOVIĆ	324
MORPHOLOGICAL PROPERTIES OF DIRECTLY SOWED AND TRANSPLANTED SWEET CORN PLANTS CULTIVATED WITH COVERING TECHNOLOGIES	
Ferenc OROSZ	328
THE INFLUENCE OF ABSORBENT AND SOIL TYPE ON GERMINATION, GROWTH AND DEVELOPMENT OF PEA GRAINS	
Vesna MILIĆ, Aleksej LUKIN, Branka GOVEDARICA, Igor ĐURĐIĆ, Tanja JAKIŠIĆ	334
YIELD OF SELECTED GENOTYPES OF SPRING PEAS (<i>Pisum sativum</i> L.) DEPENDING ON TOP DRESSING AND AGROECOLOGICAL CONDITIONS	
Igor DJURDJIC, Vesna MILIC, Vladeta STEVOVIC, Branka GOVEDARICA, Goran PERKOVIC	341
DETECTING THE BERRY SIZE RESPONSES IN TWO STRAWBERRY CULTIVARS USING DIFFERENT IRRIGATION LEVELS AND BIO-ACTIVATOR	
Eser ÇELİKTOPUZ, Burçak KAPUR, Mehmet Ali SARIDAŞ, Sevgi PAYDAŞKARGI, Bülent ÖZEKİCİ, Müge ERKAN CAN, Uğur KEKEÇ	348
VARIABILITY OF CAROTENOIDS AND TOCOPHEROLS CONTENT IN MAIZE INBRED LINES	
Snežana MLADENOVIĆ DRINIĆ, Jelena MESAROVIĆ, Natalija KRAVIĆ, Jelena SRDIĆ, Milan STEVANOVIĆ, Ana NIKOLIĆ, Violeta ANĐELKOVIĆ	354
MINERAL COMPOSITION OF TWO PEPPER CULTIVARS (<i>CAPSICUM ANNUUM</i> L) FROM SERBIA AT THREE RIPENING STAGES	
Milica LUČIĆ, Ivana SREDOVIĆ IGNJATOVIĆ, Slađana ĐURĐIĆ, Antonije ONJIA	360
EFFECT OF DIFFERENT STORAGE TEMPERATURES ON PHYSICAL STATE AND SOME INNER CONTENTS OF SWEET POTATO (<i>IPOMOEA BATATAS</i> L.) BULBS	
Nour AHADIDI, Tamas ZSOM, Katalin FEKETE, Mariann KIS KRISZTIANNE, Zoltan PAP	366
EFFECT OF DRYING ON THE CHANGE OF SUGAR CONTENT IN PLUM FRUITS	
Olga MITROVIĆ, Branko POPOVIĆ, Nemanja MILETIĆ, Aleksandar LEPOSAVIĆ, Aleksandra KORICANAC	372
VARIABILITY OF QUALITY AND RHEOLOGICAL PROPERTIES IN WINTER WHEAT UNDER THE INFLUENCE OF ECOLOGICAL FACTORS	
Kristina LUKOVIĆ, Veselinka ZEČEVIĆ, Vladimir PERIŠIĆ, Vesna PERIŠIĆ, Vera RAJIČIĆ, Kamenko BRATKOVIĆ, Mirela MATKOVIĆ STOJŠIN.....	379

VARIATION IN MORPHOLOGICAL AND CHEMICAL TRAITS IN RED AND YELLOW MINI WATERMELON	
Milica STOJANOVIĆ, Đorđe MORAVČEVIĆ, Slađana SAVIĆ, Jelena DRAGIŠIĆ MAKSIMOVIĆ, Vuk MAKSIMOVIĆ	385
INFLUENCE OF CULTIVAR, MICROBIOLOGICAL FERTILIZERS AND GROWING SEASONS ON NITRATE CONTENT IN LETTUCE	
Milica STOJANOVIĆ, Milena ŽUŽA, Ivana PETROVIĆ, Đorđe MORAVČEVIĆ, Gorica CVIJANOVIĆ, Dragosav MUTAVDŽIĆ, Slađana SAVIĆ	390
IMPACT OF PASTERIZATION ON THE QUALITY OF POMEGRANATE JUICE FROM "HICAZ" VARIETY FROM THE REGION OF NORTH MACEDONIA	
Violeta DIMOVSKA, Fidanka ILIEVA, Sanja KOSTADINOVIĆ VELIČKOVSKA, Ljupco MIHAJLOV, Biljana KOVAČEVIĆ, Elizabeta DAVITKOVSKA BARBAREEVA	396
NUTRITIVE CHARACTERISTICS AND ANTIOXIDANT ACTIVITY OF PSEUDOGRAINS	
Bojana VIDOVIĆ, Mirjana MARČETIĆ, Snežana JANKOVIĆ, Sveto RAKIĆ, Maja PAVLOVIĆ, Katarina PERIŠIĆ, Radojica RAKIĆ	401
BIODIVERSITY OF CHICKPEA: BLACK CHICKPEA	
Ali KAHRAMAN	408
SUSTAINABLE SOIL USAGE: LEGUMES AND HEAVY METALS	
Ali KAHRAMAN	412
NITROGEN FIXATION IN LEGUMES AND CHANGES IN CLIMATE	
Mustafa ONDER, Ali KAHRAMAN	416
SUSTAINABLE PESTICIDE USAGE IN FARMING LEGUMES	
Mustafa ONDER, Ali KAHRAMAN	420
BIODIVERSITY ON PASTURES: HUNTING AND SOCIO-ECONOMIC DIMENSIONS	
Serpil ONDER, Ali KAHRAMAN	423
EFFECTS OF A SUBSTRATE IMPROVEMENT AGENT ON THE VEGETATIVE GROWTH AND QUALITY OF VEGETABLE SEEDLINGS	
Zoltan PAP, Katalin FEKETE, Dzszenifer NEMETH, Levente KARDOS, Lyza Maquilan QUILONG QUILONG, Mohammed Omer MUSTAFA, Anna SZABO, Gabor BALAZS	427
EFFICACY OF MULTILAYER CROPPING ON FIG PRODUCTION IN ARID CONDITIONS OF KUWAIT	
Hani A. AL-ZALZALEH, Gladson DCRUZ	433
ASSESSMENT OF SAP FLOW TECHNIQUES AS AN AUTOMATED INDICATOR OF <i>VITIS VINIFERA</i> WATER STATUS IN THE PORTUGAL'S DOURO VALLEY	
Aureliano C. MALHEIRO, Nuno CONCEIÇÃO, José SILVESTRE, Maria Isabel FERREIRA	440
THE EFFECT OF DIFFERENT FOLIAR FERTILIZERS AND DOSAGES ON THE YIELD AND SEED QUALITY CHARACTERISTICS OF DURUM WHEAT	
Kyriakos D. GIANNOULIS, Eleni WOGIATZI-KAMWOUKOU, Niki C. ASIMAKI, Ilias PLATIS, Dimitrios KOUTLIS	444

THE EFFECT OF LIMING ON THE ALUMINIUM CONTENT IN A WHEAT ROOT

Nebojša GUDZIĆ, Gordana ŠEKULARAC, Vera RAJIČIĆ, Slaviša GUDZIĆ, Miroljub AKSIĆ, Dragan GRČAK, Aleksandar ĐIKIĆ, Jasmina KNEŽEVIĆ451

DETERMINATION OF VITAMIN C CONTENT IN DIFFERENT EXTRACTS OF THE *ALCHEMILLA VULGARIS* L.

Jelena MLADENOVIĆ, Duško BRKOVIĆ, Milena ĐURIĆ, Ljiljana BOŠKOVIĆ-RAKOČEVIĆ, Jasmina ZDRAVKOVIĆ, Nenad PAVLOVIĆ456

GERMINATION OF *CAPPARIS SPINOSA* L. SEEDS UNDER DIFFERENT DORMANCY BREAKING TREATMENTS

Marija MARKOVIĆ, Mihailo GRBIĆ, Dragana SKOČAJIĆ, Matilda ĐUKIĆ, Danijela ĐUNISIJEVIĆ-BOJOVIĆ460

NITROGEN FERTILIZATION AND SOWING DENSITY INFLUENCE ON WINTER WHEAT YIELD AND YIELD COMPONENTS

Vladimir AČIN, Milan MIROSAVLJEVIĆ, Goran JAĆIMOVIĆ, Bojan JOCKOVIĆ, Ljiljana BRBAKLIĆ, Dragan ŽIVANČEV, Sonja ILIN465

INFLUENCE OF SUPERABSORBENT „TVERDAYA VODA” ON MORPHOLOGICAL CHARACTERISTICS OF POTATO

Saša LALIĆ, Vesna MILIĆ, Aleksej LUKIN, Branka GOVEDARICA, Igor DJURDJIC, Andrija TOMIĆ471

NUTRITION VALUES OF HULLED AND NAKED OATS AND BAKING QUALITY OF WHEAT-OAT COMPOSITE FLOURS

Soňa GAVURNÍKOVÁ, Michaela HAVRLETOVÁ479

DETERMINATION OF PHYSICAL AND CHEMICAL PARAMETERS OF SELECTED VARIETIES OF BUTTERNUT SQUASH (*CUCURBITA MOSCHATA* DUCH. EX POIR.)

Alena ANDREJIOVÁ, Alžbeta HEGEDŮSOVÁ, Adriána MAŤOVÁ, Ondrej HEGEDŮS, Andrea VARGOVÁ486

IDENTIFICATION OF S-ALLELES IN SOME INDIGENOUS SWEET CHERRY GENOTYPES GROWN IN OHRID REGION

Slađana MARIĆ, Sanja RADIČEVIĆ, Melpomena POPOVSKA, Viktor GJAMOVSKI, Nebojša MILOŠEVIĆ493

RESEARCH ON IMPROVING TOMATO GROWTH ON PERLITE SUBSTRATE BY HORSE URINE AND BENEFITS FOR PLANT RESISTANCE TO PATHOGENS

Carmen COARFĂ, Sorin MATEI, Gabi-Mirela MATEI, Ovidiu Ionuț JERCA, Elena Maria DRĂGHICI501

PRELIMINARY RESULTS ON USING CAPILLARY DYNAMOLYSIS IN ASSESSING THE EFFECT OF STRUCTURED WATER ON CUCUMBER PLANTS

Florin ENACHE, Sorin MATEI, Gabi-Mirela MATEI, Ionuț Ovidiu JERCA, Elena Maria DRĂGHICI507

HARVEST TIME IMPACT ON SHATTERING HABIT AND MILLING QUALITY OF RICE CULTIVARS

Alireza ALLAMEH513

ANALYSIS AND CHARACTERISTICS OF THE NEW VIRGINIA HYBRID LINES IN CMS FORM	
Ilija RISTESKI, Karolina KOCOSKA	520
EVALUATION OF ANDROGENIC COMPETENCE OF DIFFERENT PEPPER, TOMATO AND EGGPLANT GENOTYPES	
Marija POCKOVSKA, Fidanka TRAJKOVA, Liljana KOLEVA GUDEVA	525
SOIL PROPERTIES AND QUALITY GRASSLANDS OF THE MUNICIPALITY OF IVANJICA	
Snežana ANĐELKOVIĆ, Tanja VASIĆ, Snežana BABIĆ, Mirjana PETROVIĆ, Zoran LUGIĆ, Vladimir ZORNIĆ, Goran JEVTIĆ	531
YIELD AND CONTENT OF STARCH AND PROTEIN IN THE SEED OF THE QUINOA GENOTYPES PUNO AND TITICACA	
Slađana SAVIĆ, Borisz CZEKUS, Ivana PETROVIĆ, Milena MARJANOVIĆ, Zorica JOVANOVIĆ, Radmila STIKIĆ	538
BOTANICAL COMPOSITION AND FORAGE QUALITY OF NATURAL GRASSLANDS OF PEŠTER HIGHLANDS	
Snežana BABIĆ, Zoran LUGIĆ, Dejan SOKOLOVIĆ, Mirjana PETROVIĆ, Vladimir ZORNIĆ, Snežana ANDJELKOVIĆ, Djordje LAZAREVIĆ	543
EFFECT OF GROWTH STAGE ON MINERAL CONCENTRATION IN THE TOP ALFALFA AND DRY MATTER YIELD	
Jordan MARKOVIĆ, Dragan TERZIĆ, Tanja VASIĆ, Snežana ANĐELKOVIĆ, Mirjana PETROVIĆ, Dragoslav ĐOKIĆ, Snežana BABIĆ	549
EFFICIENCY OF EQUIPMENT FOR CLEANING OF THE NATURAL RED CLOVER (<i>Trifolium pratense</i> L.) SEEDS	
Dragoslav ĐOKIĆ, Rade STANISAVLJEVIĆ, Dragan TERZIĆ, Jasmina MILENKOVIĆ, Tanja VASIĆ, Jordan MARKOVIĆ, Ranko KOPRIVICA	555
GRAIN YIELD, YIELD COMPONENTS, MACRO AND MICROELEMENTS VARIATION ANALYSIS IN WHEAT GROWN ON SOLONETZ SOIL	
Miodrag DIMITRIJEVIĆ, Sofija PETROVIĆ, Borislav BANJAC, Petar SEKULIĆ	561
MINERAL ELEMENTS TRANSLOCATION AND RELATIVE YIELD AND YIELD COMPONENTS TOLERANCE IN WHEAT GROWN ON SOLONETZ SOIL	
Sofija PETROVIĆ, Miodrag DIMITRIJEVIĆ, Borislav BANJAC, Petar SEKULIĆ	568
THE EFFECTIVENESS OF SOIL TILLAGE SYSTEMS IN MAIZE CULTIVATION UNDER VARIABLE METEOROLOGICAL CONDITIONS OF CENTRAL SERBIA	
Milena SIMIĆ, Vesna DRAGIČEVIĆ, Branka KRESOVIĆ, Dušan KOVAČEVIĆ, Željko DOLIJANOVIĆ, Milan BRANKOV	575
THE INFLUENCE OF PLANT CUTTING ON GRAIN YIELD TRAITS IN MAIZE	
Jelena VANČETOVIĆ, Dragana IGNJATOVIĆ-MICIĆ, Sofija BOŽINOVIĆ, Ana NIKOLIĆ, Dejan DODIG, Vesna KANDIĆ, Olivera ĐORĐEVIĆ MELNIK	581
TROPHIC STRUCTURE OF THE NEMATODE COMMUNITY IN MONOCULTURES OF CARROTS (<i>DAUCUS CAROTA</i> SUBSP. <i>SATIVUS</i>)	
Liljana KOLEVA, Deria ADEM	587
MICROMORPHOLOGICAL AND BIOCHEMICAL ASPECTS IN <i>MENTHA x PIPERITA</i> L. FOR INDUSTRIAL APPLICATIONS	

Jailawi Asmaa ALI, Monica Luminița BADEA, Vlad POPA, Sorin MATEI, Gabi-Mirela MATEI, Oana Crina BUJOR, Elena Maria DRĂGHICI,.....	594
STUDYING THE ACCLIMATIZATION AND AGRICULTURAL PECULIARITIES OF EARLY SPECIES OF TANGERINE INTRODUCED FROM JAPAN IN CONDITIONS OF ADJARA	
Rezo JABNIDZE, Mirza KURSHUBADZE, Mamuka TURMANIDZE, Nana JABNIDZE.....	600
GENERAL COMBINING ABILITIES OF ELITE MAIZE INBRED LINES FOR YIELD AND IMPORTANT TRAITS	
Nikola GRČIĆ, Ana STEPANIĆ, Marko MLADENović, Milan STEVANOVIĆ, Jovan PAVLOV, Zoran ČAMDŽIJA	605
ESTIMATION OF YIELD STABILITY OF ZP MAIZE HYBRIDS USING DIFFERENT STATISTICAL MODELS	
Jovan PAVLOV, Nenad DELIĆ, Milan STEVANOVIĆ, Zoran ČAMDŽIJA, Gordana BRANKOVIĆ, Ana STEPANIĆ, Marija KOSTADINOVIĆ	610
EFFECT OF DIFFERENT SIZES OF (PRE BASIC SEEDS) MINITUBER ON PLANT GROWTH AND SEED YIELD OF POTATO	
Shyama Nadeeka HETTIARACHCHI, Nilusha AMARASINGHA, George Chandra BABU, Kanagarathnam DAYANI AND RATHNAKUMARA.....	614
INFLUENCE OF SOIL MOISTURE REGIME ON ALLUVIAL FOREST HABITAT	
Tanja JAKISIC, Vesna NIKOLIC JOKANOVIC, Radovan SAVIC, Ljubomir LETIC, Marko GUTALJ	619
EFFECT OF NITRIC OXIDE (NO) APPLICATION ON THE DEVELOPMENT OF PEPPER PLANT UNDER DROUGHT STRESS	
Fikret YASAR, Ozlem UZAL.....	626
OLIVE PRODUCTION AND TRAITS OF MAIN CULTIVARS GROWN SOUTHEAST ANATOLIAN PART OF TURKEY	
Bekir Erol AK, Ebru SAKAR, Sehnaz KORKMAZ.....	631
THE TRAITS OF PISTACHIO ROOTSTOCKS AND THEIR IMPORTANCE	
Bekir Erol AK.....	639
EFFECTS OF DIFFERENT SOIL TYPES ON SOME FRUIT PHYSICAL TRAITS OF 'KETEN GOMLEGI' PISTACHIO CULTIVAR	
Ahmet CELIK, Bekir Erol AK.....	646
ECO-FRIENDLY WATER-RETAINING AGENTS AS THE BASIS FOR POTATO AGROTECHNOLOGY (Black-Earth Region, Russia)	
Alexey LUKIN, Olga MARAEVA, Tatyana NEKRASOVA, Nadezhda PODLESNYKH, AleksanderKASHIRIN, VyacheslavKUZNETSOV, Vladimir SELEMENEV	652
CHEMICAL COMPOSITION AND YIELD OF DILL AS AFFECTED BY METALLURGICAL SLAG AND ORGANIC FERTILIZER APPLIANCE TO MARGINAL SOIL	
Aleksandra STANOJKOVIĆ-SEBIĆ, Radmila PIVIĆ, Zoran DINIĆ, Dobrivoj POŠTIĆ, Jelena MAKSIMOVIĆ	658
ASSESMENT OF THE POSSIBILITIES FOR ALTERNATIVE WEED CONTROL IN SOYBEAN	

Bogdan NIKOLOV, Slaveya PETROVA, Ekaterina VALCHEVA, Irena GOLUBINOVA, Plamen MARINOV-SERAFIMOV	664
ALLELOPATIC EFFECT OF FIVE WEED SPECIES ON SEED GERMINATION OF SORGHUM CROPS	
Bogdan NIKOLOV, Slaveya PETROVA, Ekaterina VALCHEVA, Irena GOLUBINOVA, Plamen MARINOV-SERAFIMOV	671
IN-DEPTH BRAIN PHOSPHOPROTEOME STUDY REVEALS NEUROBIOLOGICAL UNDERPINNINGS FOR FORAGER HONEYBEE WORKERS (<i>APIS MELLIFERA LIGUSTICA</i>)	
Haitham RAMADAN, Jianke LI.....	677
BEHAVIOR OF YOUNG CONSUMERS REGARDING FLAVORED WINES - CASE STUDY: RETSINA AND VERMOUTH	
Vasiliki - Foteini MPAKABELLOU, Efthymia OIKONOMOU, Vasileios GREVENIOTIS, Evangelia SIOKI, Elisavet BOULOUMPASI, Athanasios MANOURAS	688
ASSESSING STABILITY IN MAIZE	
Vasileios GREVENIOTIS, Stylianos ZOTIS, Evangelia SIOKI, Constantinos G. IPSILANDIS	693
DEVELOPMENT OF CHANGES OF LACTIC ACID AND COLOR CONTENT IN HARVEST SEASON FROM ELDERBERRY (<i>SAMBUCUS NIGRA L.</i>)	
Zsuzsanna PALÁGYI, Diána FURULYÁS, Mónika STÉGER-MÁTÉ	697
REVALUATION OF NATIVE CORN TO FACE ENVIRONMENTAL CHANGE	
José Sergio BARRALES-DOMÍNGUEZ; Edgar BARRALES-BRITO.....	702
PRODUCTIVITY OF RED CLOVER-ITALIAN RYEGRASS MIXTURES ON ACIDIC SOIL DEPENDING OF THE LIMING	
Dalibor TOMIĆ, Đorđe LAZAREVIĆ, Vladeta STEVOVIĆ, Dragan ĐUROVIĆ, Milomirka MADIĆ, Vladimir ZORNIĆ, Jasmina KNEŽEVIĆ	707
LENGTH OF THE ONE-YEAR-OLD BRANCHES AND CATKIN POSITION ON THEM AFFECT QUANTITY AND MORPHOLOGICAL CHARACTERISTICS OF STAMINATE FLOWERS IN WALNUT	
Slađana JANKOVIĆ, Jelena STANKOVIĆ, Dragan JANKOVIĆ, Zoran NIKOLIĆ, Gorica PAUNOVIĆ, Bratislav ĆIRKOVIĆ	713
THE EFFECT OF TEMPERATURE ON POLLEN GERMINATION OF TWO WALNUT CULTIVARS	
Slađana JANKOVIĆ, Jelena STANKOVIĆ, Dragan JANKOVIĆ, Zoran NIKOLIĆ, Gorica PAUNOVIĆ, Bratislav ĆIRKOVIĆ.....	719
THE EFFECT OF CYTOPLASMATIC MALE STERILITY ON YIELD AND YIELD COMPONENTS OF MAIZE INBRED LINES	
Milan STEVANOVIĆ, Snežana MLADENOVIĆ DRINIĆ, Jovan PAVLOV, Nikola GRČIĆ, Danijela RISTIĆ, Milica NIKOLIĆ, Iva SAVIĆ	725
QUALITATIVE ANALYSIS OF SUNFLOWER SEEDING IN REDUCED SOIL TILLAGE USING PRECISION AGRICULTURE SYSTEM	
Stanko OPARNICA, Vladimir VIŠACKI, Jan TURAN, Ondrej PONJIČAN, Aleksandar SEDLAR, Rajko BUGARIN.....	730
THE EFFECT OF DIFFERENT FERTILIZER APPLICATIONS ON PLANT DEVELOPMENT AND FLOWERING OF DEMRE PEPPER	

Fikret YASAR, Ozlem UZAL.....	738
BIOCHEMICAL STRESS MARKERS OF PLANTS IN CONDITIONS OF FOREST AND PARK ECOSYSTEMS	
Oksana KLYACHENKO, Artur LIKHANOV, Nataliya MIROSHNYK, Igor TESLENKO	742
2. PLANT PROTECTION AND FOOD SAFETY	752
PHENOLIC COMPONENTS AND ANTIOXIDANT ACTIVITIES IN VARIOUS TYPES OF CARROT EXTRACTS	
Jelena MLADENović, Ljiljana BOŠKOVIĆ-RAKOČEVIĆ, Milena ĐURIĆ, Jasmina ZDRAVKOVIĆ, Nenad PAVLOVIĆ, Marijana DUGALIĆ	753
CHANGES IN GERMINATION AND PRIMARILY GROWTH OF THREE CULTIVARS OF FENNEL UNDER APPLICATION OF DIFFERENT DIATOMITE TREATMENTS	
Mohamad Hesam SHAHRAJABIAN ^a , Wenli SUN ^a , Qi CHENG ^a	757
ANISE SEED GERMINATION AND PRIMARILY GROWTH UNDER VARIOUS TREATMENTS OF GIBBERELIC ACID, BENZYLADENINE AND KINETIN	
Mohamad Hesam SHAHRAJABIAN ^a , Wenli SUN ^a , Qi CHENG ^a	763
INTERCROPPING AND ORGANIC FERTILIZERS WITH COMPARATIVE ADVANTAGES IN SUSTAINABLE AGRICULTURE FOR MORE STABLE AGRICULTURAL SYSTEM	
Mohamad Hesam SHAHRAJABIAN, Wenli SUN , Mehdi KHOSHKHARAM, Peiman ZANDI, Qi CHENG	770
PROSPECTIONS FOR FOOD BASED LURES IN MASS TRAPPING OF <i>BACTROCERA DORSALIS</i> ON <i>CHRYSOPHYLLUM ALBIDUM</i> IN OSUN STATE, NIGERIA	
Juliana Amaka UGWU	777
PHYTOCHEMICAL CHARACTERISTICS OF DIFFERENT MALTS AND POSSIBILITIES FOR THEIR APPLICATION IN FUNCTIONAL BEVERAGES	
Vesela SHOPSKA, Mina DZIVODEROVA-ZARCHEVA, Kristina IVANOVA, Petar NEDYALKOV, Rositsa DENKOVA-KOSTOVA, Maria KANEVA, Georgi KOSTOV	785
CONTROL TRIALS OF ANABOLIC HORMONE RESIDUES IN TISSUES OF WILD AND FARMED NILE TILAPIA IN EGYPT	
Mohamed HAMADA, Reda KHADER, Abdel Rahman EL-BAGORY	791
LEGAL REQUIREMENTS FOR PREPARATION AND PRODUCTION OF FOOD IN BOSNIA AND HERZEGOVINA WITH SPECIAL REFERENCES ON THE ENTITY OF THE REPUBLIC OF SRPSKA	
Saša LALIĆ, Dragan VUJADINOVIĆ.....	797
EFFECT OF DIFFERENT THICKENING AGENTS ON FREEZE-THAW STABILITY OF FOOD SYSTEMS WITH MODIFIED MAIZE STARCH	
Mina DZHIVODEROVA-ZARCHEVA, Kremena NIKOVSKA, Raina HADJIKINOVA, Vesela SHOPSKA	807
TOXIGENIC POTENTIAL OF <i>ASPERGILLUS PARASITICUS</i> ISOLATES ORIGINATING FROM MAIZE GRAIN IN SERBIA	
Milica NIKOLIĆ, Iva SAVIĆ, Ana OBRADOVIĆ, Milan STEVANOVIĆ, Jovan PAVLOV, Marko JAUKOVIĆ, Slavica STANKOVIĆ.....	813

COMPARATIVE EVALUATION OF MOLECULAR METHODS USED FOR FUSARIUM STRAINS IDENTIFICATION IN THE WEST PART OF ROMANIA Sorina POPESCU, Dorica BOTAU, Aurica BOROZAN, Ioan SARAC, Oana-Maria BOLDURA.....	818
EVALUATION OF SPRAY QUALITY USING WATER SENSITIVE PAPERS AND SIMULATED TREE CANOPY Reza TABATABAEKOLOR, Behrooz MOKHTARPOUR, Yahya ABTALI	824
TESTING ANTIBACTERIAL ACTIVITIES OF <i>SEMPERVIVUM TECTORUM</i> Vesna KALABA, Željko SLADOJEVIĆ, Željka MARJANOVIĆ BALABAN, Dragana KALABA	830
EFFECTS OF ZINC OXIDE NANOPARTICLES ON <i>SOLANUM LYCOPERSICUM</i> L. AND VIABILITY OF <i>TENEBRIO MOLITOR</i> (TENEBRIONIDAE) Ivan RANKIĆ, Marek DVORÁK, Bořivoj KLEJDUS, Helena ŠTUŘÍKOVÁ, Dalibor HÚSKA.....	836
VEGETABLE OILS: DO THEY HAVE ANTIOXIDANT ACTIVITY? Raisa IVANOVA, Dina ELISOVETCAIA, Pavel TATAROV	843
HAZARDS RELATED TO FOOD SAFETY Yasin AKKEMİK.....	850
THE EFFECTS OF PROBIOTICS ON PUBLIC HEALTH Yasin AKKEMİK.....	856
THE INFLUENCE OF ABIOTIC FACTORS IN DEVELOPMENT OF CODLING MOTH AND ITS NATURAL ENEMIES IN SOUTHERN SERBIA Katerina NIKOLIĆ, Ana SELAMOVSKA, Slaviša GUDŽIĆ, Zoran NIKOLIĆ, Dragan GRČAK, Milosav GRČAK.....	860
EFFECT OF MODE PACKAGING AND STORAGE ON SENSORY PROPERTIES AND COLORS IN LETTUCE Danijela VASKOVIĆ, Midhat JAŠIĆ	866
LABORATORY GERMINATION AND EPIPHYTIC MYCOPHLORA OF VARIOUS GENOTYPES OF SORGHUM (<i>SORGHUM BICOLOR</i> (L.) MOENCH) Mariya KASCHIEVA, Krasimira TANOVA	874
EFFICIENCY AND THE POSSIBILITY OF USING ZEOLITE AND APATITE IN PURIFYING WATER FOR IRRIGATION AND REMEDIATION OF CONTAMINATED SOIL Mirko GRUBIŠIĆ, Ljiljana BOŠKOVIĆ-RAKOČEVIĆ, Goran DUGALIĆ, Jelena PETROVIĆ, Marija PETROVIĆ, Jelena MILOJKOVIĆ, Marija KOJIĆ	880
USAGE OF PARSLEY POWDER AS AN ALTERNATIVE CURING AGENT IN FERMENTED SUCUK PRODUCTION Ali Samet BABAĞLU, Mustafa KARAKAYA.....	886
BIOCONTROL OF STRAWBERRY GREY MOLD USING PEPPER EXTRACTS Lina ŠERNAITĖ, Alma VALIUŠKAITĖ, Neringa RASIUKIČIŪTĖ, Edita DAMBRAUSKIENĖ, Pranas VIŠKELIS.....	893
MONITORING OF THE SAFETY OF WILD -GROWING FRUITS FROM DIFFERENT REGIONS OF KAZAKHSTAN Gulnar AIDARKHANOVA, Raya AIDARKULOVA, Talgat ABZHANOV, Zhumagul BORANBAI	899

COMPARING OF PREEMERGENT WEED CONTROL TECHNOLOGIES IN SOYBEAN (GLYCINE MAX) György PÁSZTOR, Bálint VÁRADI, Veronika DOBSZAI-TÓTH, Erzsébet NÁDASY	905
CONTENT OF Cu, Zn, Co, Ni, Cr IN SOIL AND FRUITS OF APPLE AND PLUM Mira MILINKOVIĆ, Svetlana M. PAUNOVIĆ, Milena ĐORĐEVIĆ, Jelena TOMIĆ, Žaklina KARAKLAJIĆ STAJIĆ, Dragana VRANIĆ	910
EFFECTS OF TEA WOOD ESSENTIAL OIL ON <i>TRIBOLIUM CONFUSUM</i> DU VAL. AND <i>ORYZAEPHILUS SURINAMENSIS</i> L. Dragana BOŠKOVIĆ.....	917
THE EFFECT OF SOME CHEMICAL ADDITIVES ON THE FOAMING PERFORMANCE OF THE PASTEURIZED LIQUID EGG WHITE Mehmet Musa ÖZCAN, Hatice Kübra YAVUZ.....	923
CHEMICAL PROTECTION OF WINTER WHEAT AND ITS ENVIRONMENTAL IMPACT IN DIFFERENT SOIL TILLAGE SYSTEMS Małgorzata HOLKA, Jerzy BIENKOWSKI	929
ANTIBACTERIAL ACTIVITY OF PROPOLIS EXTRACTS FROM GREECE AND REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA) Vesna KALABA , Tanja ILIĆ, Dragana KALABA, Željko SLADOJEVIĆ, Bojan GOLIĆ	935
INFLUENCE OF INOCULATION OF <i>Q. CERRIS</i> SEEDLINGS WITH A ROOT PATHOGEN <i>P. PLURIVORA</i> ON THE PERFORMANCE OF <i>L. DISPAR</i> LARVAE UNDER EXTREMELY ELEVATED CO₂ LEVEL CONDITIONS Slobodan MILANOVIĆ, Jovan DOBROSAVLJEVIĆ, Nemanja KAŠIĆ, Marija POPOVIĆ, Ivan MILENKOVIĆ, Stefan BOJIĆ, Čedomir MARKOVIĆ	943
DIFFERENT ASPECTS OF NON-STANDARD FOLIAR FERTILIZERS BASED ON AMINO ACIDS, PHYTOHORMONES AND PLANT EXTRACTS Bogdan NIKOLIĆ, Hadi WAISI, Vesna DRAGIČEVIĆ, Sanja ĐUROVIĆ, Bojan JANKOVIĆ, Maja PAGNAZZO, Vladan JOVANOVIĆ	949
COMPARISON OF THE BIOACTIVITY OF PROPOLIS EXTRACTED BY OLIVE OIL PRODUCED BY DIFFERENT PRODUCTION PROCESSES Sibel SİLİCİ	955
NUTRITIONAL CONTENT OF BEE BREAD (PERGA) FROM NEW DISCOVERED HONEYBEE PRODUCTS Sibel SİLİCİ	961
PRESENCE OF DEOXYNIVALENOL IN BREAD IN SERBIA DURING 2018-2019 Marko M. JAUKOVIĆ, Veselinka M. ZEČEVIĆ, Milica J. NIKOLIĆ	966
FUNGAL BIOMASS AND MYCOTOXIN IN KERNELS OF <i>AVENA L.</i> Svetlana ŠLIKOVÁ, Martina HUDCOVICOVÁ, Edita GREGOVÁ, Peter HOZLÁR	970
CONTROL OF APHIDIDAE IN CHRYSANTHEMUM UNDER GREENHOUSE PRODUCTION Slavica VUKOVIĆ, Sanja LAZIĆ, Dragana ŠUNJKA, Antonije ŽUNIĆ, Dragana BOŠKOVIĆ	977
APPLICATION OF CHITOSAN-BASED COATING IN LEMON FRUIT CONSERVATION S. Jafar HASHEMI, Ebrahim TAGHINEZHAD, Reza TABATABAEE	981

ANTIMICROBIAL ACTIVITY OF PGPR ACTINOBACTERIA

Lamia AOUAR, Ines BOUKELOUL, Abderrahmane BENADJILA986

INVESTIGATION OF THE OXIDATION PRODUCTS OF THE OILS OF THE LATEST NON-OILY SUNFLOWER HYBRID SEEDS

Tanja LUŽAIĆ, Ranko ROMANIĆ, Nada GRAHOVAC, Nada HLADNI, Snežana KRAVIĆ, Zorica STOJANOVIĆ.....993

THE EFFECTS OF WATER EXTRACTS OF XERANTHEMUM CYLINDRACEUM SIBTH. ET SM. ON SEED GERMINATION OF THREE WEED SPECIES

Vladan JOVANOVIĆ, Miloš GALOVIĆ, Bogdan NIKOLIĆ, Ivana DRAGIĆEVIĆ, Hadi WAISI, Zlatko GIBA999

BIOCONTROL POTENTIAL OF *TRICHODERMA HARZIANUM* AGAINST ROT CAUSING FUNGI OF WHITE YAM (*DIOSCOREA ROTUNDATA* POIR) TUBERS

Victor Iorungwa GWA., Alphonsos Okechukwu NWANKITI 1006

INFLUENCE OF MACERATION TIME OF GRAPE POMACE CABERNET SAUVIGNON ON EXTRACTION KINETICS OF SOME POLYPHENOLS AND ANTI-DPPH RADICAL ACTIVITY OF WINES

Nikolina LIŠOV, Uroš ČAKAR, Aleksandar PETROVIĆ, Ljiljana GOJKOVIĆ-BUKARICA 1015

INTERSPECIFIC IDENTIFICATION OF SOME APHID SPECIES BASED ON THE MITOCHONDRIAL CYTOCHROME OXIDASE I (mtCOI) PARTIAL GENE

Samir ALI AROUS, Yamina GUENAOUI 1019

FUNGAL PATHOGENS OF BIRDSFOOT TREFOIL (*Lotus corniculatus* L.) IN SERBIA

Tanja VASIĆ, Vesna KRNJAJA, Jordan MARKOVIĆ, Snežana ANDJELKOVIĆ, Mirjana PETROVIĆ, Aleksandra LEPOSAVIĆ, Dragan TERZIĆ 1025

PRESENCE OF VIRUSES IN THE POPULATION OF GRAPEVINE CULTIVAR "PROKUPAC" (*Vitis vinifera* L.) IN RASINA DISTRICT, SERBIA

Sandra MILETAKOVIC, Sladjan STANKOVIC, Dragan RAHOVIC, Slobodan KRNJAJIC, Ivana BAKIC, Radomir JOVANOVIC..... 1030

PHYTOCHEMICAL AND BIOLOGICAL SCREENING OF APIACEAE THAPSIA GARGANICA L.

Fatma ACHEUK, Khemais ABDELLAOUI, Wassima LAKHDARI, Abderrahmène DEHLIZ, Nora CHAHBAR, Messaouda BELAID, Lamia YALAOUI, Hadjer BOUKAHAL..... 1033

AMARANTH GRAIN AS ENRICHING INGREDIENT FOR BAKERY TECHNOLOGY

Anastasiia STAKHURLOVA, Natalia DERKANOSOVA, Irina PONOMARYOVA 1042

EXAMINATION OF VITAMIN C IN FEED AND MILK

Julijana TOMOVSKA, Dijana BLAZEKOVIĆ-DIMOVSKA, Ilmije VLASAKU 1048

STUDY OF BIOLOGICAL EFFECTIVENESS AND RESIDUE DYNAMICS OF USED NEONICOTINOIDS AND PYRETHROIDS ON CEREALS

Ilya KASATOV, Anton POLIKARPOV, Yuriy SAVUSHKIN, Elena TESTOVA, Anastasia FILATOVA 1056

BIOCHEMICAL CHARACTERISTICS OF MEDICINAL RAW MATERIALS OF MYRTUS COMMUNIS L. IN CONDITIONS OF THE SOUTHERN COAST OF THE CRIMEA	
Yuri PLUGATAR, Ekaterina BAKOVA, Oksana SHEVCHUK, Anfica PALIY, Nadezhda BAKOVA	1061
ANALYSIS OF FUNGICIDES BOSCALID AND PYRACLOSTROBIN RESIDUES IN LETTUCE	
Sanja LAZIĆ, Dragana ŠUNJKA, Dragana BOŠKOVIĆ, Monika TOMŠIK, Denis GRNČA, Slavica VUKOVIĆ, Antonije ŽUNIĆ.....	1067
INFLUENCE OF BIOTIC STRESS ON TURKEY OAK SEEDLINGS IN ELEVATED CO₂ CONDITIONS	
Slobodan MILANOVIĆ, Jovan DOBROSAVLJEVIĆ, Nemanja KAŠIĆ, Marija POPOVIĆ, Ivan MILENKOVIĆ, Stefan BOJIĆ, Čedomir MARKOVIĆ	1072
MACRO AND MICRO ELEMENT CONTENTS OF SOME HERB AND CONDIMENTS	
Nurhan USLU, Mehmet Musa ÖZCAN, Mustafa HARMANKAYA, Nesim DURSUN, Mustafa Mete ÖZCAN, Durmuş Ali CEYLAN, Fatma GÖKMEN	1078
EFFECT OF SALICYLIC ACID APPLICATION ON PHENYLALANINE AMMONIA-LYASE ACTIVITY AND TOTAL PHENOLIC CONTENT IN WHEAT (<i>Triticum aestivum</i> L.) AND BUCKWHEAT (<i>Fagopyrum esculentum</i> MOENCH) PLANTS UNDER CADMIUM STRESS	
Yana KAVULYCH, Myroslava KOBYLETSKA, Olga TEREK.....	1086
CORNERSTONE OF STRATEGY AIMED AT CREATION OF RESISTANT VARIANTS OF CARROT (<i>DAUCUS CAROTA</i> L.) TO WHITE AND GRAY ROT PATHOGENS AT THE FEDERAL SCIENTIFIC VEGETABLE CENTER (FGBNU FNCO, RUSSIA)	
Irina ENGALYCHEVA, Elena KOZAR, Alexandr USHAKOV	1092
3. ORGANIC AGRICULTURE	1099
DEPENDENCE OF GRAIN YIELD OF SPELT ON LOCALITIES AND DIFFERENT TYPES OF FERTILIZER	
Željko DOLIJANOVIĆ, Snežana OLJAČA, Dušan KOVAČEVIĆ, Zoran JOVOVIĆ, Srđan ŠEREMEŠIĆ, Nemanja GRŠIĆ	1100
INFLUENCE OF SEEDING RATE ON COMMON VETCH-WHEAT FODDER CROP MIXTURE PERFORMANCE UNDER ORGANIC MANAGEMENT	
Dubravko MAČEŠIĆ, Matija VRBANČIĆ, Sanja SIKORA, Zlatko SVEČNJAK, Luboš VOZAR, Darko UHER.....	1106
ORGANIC AGRICULTURE IN CENTER REGION OF PORTUGAL: THE BEIRA LITORAL AND LIS VALLEY DISTRICT CASE STUDY	
Maria de Fátima OLIVEIRA, Jorge BULHA, José MENDES, Susana FERREIRA, Rosa GUILHERME, António DINIS FERREIRA, Rodolfo BERNABÉU, José Manuel GONÇALVES, Francisco GOMES DA SILVA.....	1111
CORRELLATION BETWEEN MULCHING, MYCORRHIZA FUNGI, AND OTHER PARAMETERS IN LETTUCE IN TWO FARMING SYSTEMS	
Katalin FEKETE, Rozália BALASSA, Nour ALHADIDI, Barbara FERSCHL, Zita SZALAI, Zoltán PAP.....	1117

KNOWLEDGE OF THE ECONOMIC EFFECT OF BIOLOGICAL METHODS APPLICATION IN AGRICULTURE	
Angel SAROV	1123
SOME ASPECTS OF THE ORGANIC FOOD MARKET IN SERBIA	
Tatjana BRANKOV, Stanislav ZEKIC, Marija JEREMIC, Bojan MATKOVSKI	1129
AGRICULTURAL UTILISATION OF BIOWASTES AND BIODEGRADABLE WASTES	
Monika JAKUBUS	1135
THE USE OF FUNGI OF THE GENUS TRICHODERMA TO REDUCE THE INFECTIOUS BACKGROUND OF THE SOIL	
Anna MARKAROVA, Maria MARKAROVA, Sergey NADEZHKIN, Oleg RAZIN	1141
ISOLATION, IDENTIFICATION AND PERFORMANCE OF PHOSPHATE-SOLUBILIZING BACTERIA FROM WARM-WATER FISH POND IN IRAN	
Nemat MAHMOUDI, Mostafa ARMANDEH, Alireza FALLH NOSRATABAD, Blažo LALEVIC	1147
ORGANIC BEEKEEPING DEVELOPMENT IN LATVIA	
Ligita MELECE, Ilze SHENA	1153
4. ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT	1160
ESTIMATION OF CLIMATE CHANGE IMPACTS ON EVAPOTRANSPIRATION BY USING DIFFERENT METHODS	
Muhammet AZLAK, Levent ŞAYLAN	1161
PHOSPHORUS, POTASSIUM AND NITROGEN ABUNDANCE IN SOIL OF DOLJEVAC MUNICIPALITY	
Milosav GRČAK, Dragan GRČAK, Dragana GRČAK, Miroљjub AKSIĆ, Slaviša GUDŽIĆ, Katerina NIKOLIĆ, Vera RAJIĆIĆ	1169
ABUNDANCE OF POTASSIUM AND PHOSPHORUS IN AGRICULTURAL SOIL OF THE MUNICIPALITIES AND THE CITY OF NIŠ	
Milosav GRČAK, Dragan GRČAK, Dragana GRČAK, Miroљjub AKSIĆ, Slaviša GUDŽIĆ, Katerina NIKOLIĆ, Vera RAJIĆIĆ	1174
FOOD WASTE IN PORTUGAL– A PUBLIC POLICY WITH THE COMMITMENT OF ALL SOCIETY	
Nuno Vieira E BRITO, Paula BICO, Carlos BRANDAO	1180
HARMONIZATION OF RELATIONS BETWEEN URBAN AREAS, AGROCOMPLEX AND FOREST BIOCENOSIS IN THE MANAGEMENT FUNCTION	
Saša OBRADOVIĆ, Branislav ŠARČEVIĆ, Dragana GRUJIĆ ŠARČEVIĆ, Raško STEFANOVIĆ, Milanka RADULOVIĆ, Branko PETRUJKIĆ, Nebojša POTKONJAK	1189
THE CONCENTRATIONS OF HEAVY METALS IN THE LIVER AND MUSCLE TISSUE OF THREE KINDS OF FISH DURING FIVE YEAR PERIOD	
Saša OBRADOVIĆ, Branko PETRUJKIĆ, Milanka RADULOVIĆ, Raško STEFANOVIĆ, Nebojša POTKONJAK, Milica ŽIVKOV-BALOŠ, Vladimir ŽIVKOVIĆ	1194

DIVERSITY AND DISTRIBUTION OF THE SPECIES OF GENUS <i>AMARANTHUS</i> L. 1753 IN VOJVODINA (SERBIA)	
Natasa MARIC, Sladjana PETRONIC, Zoranka MALESEVIC	1199
ROOT DISTRIBUTION OF DRIP IRRIGATED CITRUS TREES PLANTED ON RIDGE	
Uğur KEKEÇ, Bulent OZEKICI	1205
SUSTAINABILITY OF RICE PRODUCTION SYSTEMS: AGRO-ECONOMIC ANALYSIS OF <i>BAIXO MONDEGO</i> AND <i>LIS</i> IRRIGATION DISTRICTS, PORTUGAL	
Fátima OLIVEIRA, Pedro REIS, Sérgio OLIVEIRA, António JORDÃO, António RUSSO, Henrique DAMÁSIO, Rui EUGÉNIO, José PAIXÃO, Filipe MELO, Manuel NUNES, Margarida TEIXEIRA, Kiril BAHCEVANDZIEV, José M. GONÇALVES	1212
POSSIBILITY AND EFFECTIVENESS OF MICRO-ORGANISMS IN PURIFICATION OF WASTE WATER, LAGUNA AND WATERWAY	
Mirko GRUBIŠIĆ, Radislav RADOVIĆ, Ljiljana BOŠKOVIĆ-RAKOČEVIĆ, Marija PETROVIĆ, Jelena PETROVIĆ, Jelena MILOJKOVIĆ, Marija KOJIĆ	1218
THE HOUSEHOLDS BIO-WASTE MANAGEMENT ON THE EXAMPLE OF THE MALOPOLSKA REGION	
Małgorzata PINK, Dawid BEDLA, Marta CZEKAJ	1224
CONTENT OF NITROGEN IN AGROCULTURAL SOIL ON THE TERRITORY OF THE MUNICIPALITIES AND THE TOWN OF NIŠ (SERBIA)	
Dragan GRČAK, Milosav GRČAK, Dragana GRČAK, Snežana GOŠIĆ-DONDO, Miroľjub AKSIĆ, Vera ĐEKIĆ, Katerina NIKOLIĆ	1229
YIELD OF GRASSLAND BIOMASS IN SECOND SWATH AND IMPACT OF APPLIED MEASURES ON CHEMICAL REACTION OF REKULTISOL	
Nenad MALIĆ, Una MATKO, Źeljko LAKIĆ	1235
AGRICULTURE AND LAND USE: CONTEXT ANALYSIS AND FUTURE PERSPECTIVES FROM THE MEDITERRANEAN TO THE APULIA REGION	
Simona GIORDANO	1244
CHRONOSEQUENCE OF NATURAL REGENERATION IN ABANDONED MINING SITES IN THE AMAZON RAINFOREST OF MADRE DE DIOS, PERU	
Edson MORALES, Nobuyiki TANAKA, Martin PILLACA, Francisco ROMÁN-DAÑOBEYTIA, Luis E. FERNANDEZ, Miles SILMAN	1252
DISPERSION AND BIOACCUMULATION OF HEAVY METALS IN PLANTS AT THE BASIN OF WEST MACEDONIA LIGNITE CENTER, GREECE	
Lazaros TSIKRITZIS, Georgios SAMIOTIS, Konstantinos TSIOPTSIAS, Stavros THEOFANIDIS	1261
BROWNFIELDS AS ENVIRONMENTAL QUALITY INDICATOR: CASE OF LATVIA	
Velta PARSOVA, Vivita PUKITE	1267
HABITAT TYPES OF EUROPEAN IMPORTANCE ON THE JAHORINA MOUNTAIN (BOSNIA AND HERZEGOVINA)	
Natasa MARIC, Sladjana PETRONIC, Vesna TUNGUZ	1274
ANALYSIS OF DROUGHT IN NEGOTIN LOWLAND FROM THE ASPECT OF PLANT PRODUCTION	

Enika GREGORIĆ, Gordana MATOVIĆ, Vesna POČUČA	1283
INTENSITY OF OOGENESIS AND SPERMATOGENESIS IN RAINBOW TROUT (<i>Oncorhynchus mykiss</i> Wabbaum, 1792) IN VARIOUS MICROENVIRONMENTAL CONDITIONS	
Selma PILIĆ, Nadžida MLAĆO, Amela KATICA, Velija KATICA, Rizah AVDIĆ, Faruk TANDIR, Jasmin KATICA, Kenan PODŽO	1289
Fe, Cu AND Zn PRESENCE IN THE SOIL AT DIFFERENT LOCATIONS IN HERZEGOVINA REGION (BOSNIA AND HERZEGOVINA)	
Alma MIČIJEVIĆ, Aida ŠUKALIĆ, Alma LETO	1298
AGRICULTURE AND CLIMATE CHANGE IN THE REPUBLIC OF SERBIA	
Dragana DROBNJAK, Nenad KATANIC	1304
EFFECTS OF DRAINAGE AND RUNOFF ON THE PRODUCTIVITY OF THE LAGOON OF GHAR EI MELH (TUNISIA)	
Mohamed Salah ROMDHANE, Chiheb FASSATOUI, Amel BEN REJEB JENHANI	1309
MERCURY CONTENT IN AGRICULTURAL SOILS AND FIELD CROPS OF CENTRAL SERBIA	
Jordana NINKOV, Stanko MILIĆ, Jelena MARINKOVIĆ, Jovica VASIN, Branka MIJIĆ, Milorad ŽIVANOV, Dragana BJELIĆ	1314
VARIATION OF SOIL STRUCTURE IN THE FOOT AND TOE SLOPES OF MT. VUKAN, EAST-CENTRAL SERBIA	
Ljubomir B. ŽIVOTIĆ, Boško A. GAJIĆ, Branka ŽARKOVIĆ, Vesna RADOVANOVIĆ, Ljiljana NEŠIĆ, Aleksandar R. ĐORĐEVIĆ	1320
COST AND ENVIRONMENTAL EFFICIENCY IN MEASURING THE ECO-EFFICIENCY PERFORMANCE OF WINTER TRITICALE PRODUCTION	
Jerzy BIENKOWSKI, Małgorzata HOLKA	1327
IMPACT OF GRAZING ON SOIL ORGANIC MATTER AND PHYSICAL PROPERTIES OF A FLUVISOL IN NORTHWEST SERBIA	
Branka KRESOVIĆ, Boško GAJIĆ, Angelina TAPANAROVA, Snežana DRAGOVIĆ, Ranko DRAGOVIĆ, Borivoj PEJIĆ, Ljubomir ŽIVOTIĆ	1333
FOOD SECURITY IN QATAR: THE BLOCKADE OF 2017 AS AN OPPORTUNITY TOWARDS A PRODUCTIVE AND SUSTAINABLE LOCAL FOOD PRODUCTION	
Tarek BEN HASSEN, Hamid EL BILALI	1339
GEOGRAPHICAL ORIGIN OF INVASIVE PLANT SPECIES IN THE WORLD AND SERBIA	
Milan GLIŠIĆ, Vera RAŠKOVIĆ, Vera MILOŠEVIĆ, Vojislav TOMIĆ	1346
DIVERSITY AND SPECIFICITY OF BIOTA IN TUNISIAN ARTIFICIAL RESERVOIRS IN A MANAGEMENT CONTEXT	
Amel BEN REJEB JENHANI, Afef FATHALLI, Khaled GHARBI, Mohamed Salah ROMDHANE	1352
RESOURCE ASSESSMENT OF ADONIS VERNALIS IN REPRESENTATIVE NATURAL LOCALITIES IN WESTERN BULGARIA	
Ina ANEVA, Petar ZHELEV, Milena NIKOLOVA, Slavcho SAVEV	1358
CLIMATE CHANGE ADAPTATION OPTIONS IN LATVIA'S AGRICULTURE	
Ligita MELECE, Ilze SHENA	1365

CHEMICAL COMPOSITION OF FLOODPLAIN SOILS OF THE LOWER DNIESTER RIVER	
Tamara LEAH, Nicolai LEAH.....	1371
IMPACT OF <i>OLEA EUROPAEA</i> SUBSPEUROPAEA VARSYLVESTRISON ABUNDANCE AND DISTRIBUTION OF SOIL INVERTEBRATES IN NORTH ALGERIA	
Malika BOUDIAF NAIT KACI, Najet MESTAR, Massiva IOUALITENE, Maya CHERIEF	1377
CALIBRATION OF SALTMED MODEL FOR INDUSTRIAL TOMATO CULTIVATION IN CENTRAL GREECE USING A DRIP IRRIGATION SYSTEM	
Maria KOKKORA, Antonios SIOURAS, Panagiotis VYRLAS, Dimitrios KALFOUNTZOS	1383
RECYCLING OF PACKAGING MATERIALS TO OBTAIN AN OENOLOGICAL ADJUVANT	
Vito Michele PARADISO, Concetta Eliana GATTULLO, Ignazio ALLEGRETТА, Giuseppe NATRELLA, Mirella NOVIELLO, Giuseppe GAMBACORTA, Roberto TERZANO	1392
SOY-BASED VEGAN BURGER: FROM WASTE TO PRODUCTION	
Seyedmahmood RAZAVIZADEH, Alvija SALASEVICIENE, Gitana ALENCIKIENE, Lina VAICIULYTE-FUNK	1397
THE PLACE OF GREEN PRODUCTION IN ENVIRONMENTAL PROTECTION AND NATURAL RESOURCE MANAGEMENT	
Sertaç ARI, Durmuş Ali CEYLAN	1402
POTENTIALS OF PRODUCED WATER <i>BACILLUS SP.</i> FOR PHA PRODUCTION AND PLANT GROWTH PROMOTION	
Nazia JAMIL, Dur-e-SHAHWAR, Rafeya SOHAIL	1409
LITHUANIAN COMPOST QUALITY: PHYSICAL CHARACTERISTICS, NUTRIENT CONTENT, HEAVY METALS AND ORGANIC POLLUTANTS	
Karolina BARČAUSKAITĖ, Romas MAŽEIKA.....	1416
5. ANIMAL HUSBANDRY	1423
EFFECTS OF GENOTYPE AND SEX ON PRODUCTIVE PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILER CHICKENS	
Jozef BUJKO, Cyril HRNČÁR, Emília HANUSOVÁ, Jiřina ZEMANOVÁ.....	1424
THE EFFECT OF APPLIED CROSSBREEDING ON INCREASING OF MILK PRODUCTION IN GOATS	
Pascal CONSTANTIN, Ionică NECHIFOR, Costică CRISTIAN.....	1431
MICROELEMENT PROFILE ANALYSIS OF GRAPE BY-PRODUCTS FROM SLOVAKIA AND AUSTRIA	
Renata KOLLÁTHOVÁ, Ondrej HANUŠOVSKÝ, Branislav GÁLIK, Daniel BÍRO, Milan ŠIMKO, Miroslav JURÁČEK, Michal ROLINEC, R. PUNTIGAM, J.A. SLAMA, Martin GIERUS	1436
HAPLOTYPE BLOCK STRUCTURE IN THE GENOME OF SLOVAK PINZGAU CATTLE	
Radovan KASARDA, Nina MORAVČÍKOVÁ, Kristína LEHOČKÁ, Barbora OLŠANSKÁ, Ján PRIŠŤÁK, Anna TRAKOVICKÁ, Ondrej KADLEČÍK.....	1442

EFFECT OF SPRAYING SULPHUR, MAGNESIUM, ZINC AND, ON FRUITING OF SUPERIOR SEEDLESS GRAPEVINES

Mustafa H. HASHEM, Faisal. F. AHMED, Ali. A. GOBARA, Ahmed. K. ABDELAAL 1448

THE DIFFERENCES IN MACROMINERAL PROFILES IN WINE BY-PRODUCTS FROM SLOVAKIA AND AUSTRIA

Ondrej HANUŠOVSKÝ, Branislav GÁLIK, Daniel BÍRO, Milan ŠIMKO, Miroslav JURÁČEK, Michal ROLINEC, Renata KOLLÁTHOVÁ, Aljoša JANJIĆ, Christian PHILIPP, Reinhard PUNTIGAM, Julia Andrea SLAMA, Martin GIERUS..... 1455

THE CHEMICAL COMPOSITION AND QUALITY OF MEAT FROM POLISH NATIVE PIG BREEDS

Władysław MIGDAŁ, Regina KULIG, Aleksandra LEKSTON, Maria WALCZYCKA, Łukasz MIGDAŁ, Anna MIGDAŁ 1462

EFFECT OF LACTATION STAGE ON THE CONCENTRATION OF SELECTED ESSENTIAL AND TOXIC ELEMENTS IN MILK OF SHEEPS FROM AREA OF SLOVAKIA WITH SLIGHTLY DISTURBED ENVIRONMENT

Martina PŠENKOVÁ, Róbert TOMAN, Vladimír TANČIN 1468

THE CHEMICAL CONTENT OF BEEF MEAT AT THE DIFFERENT FEEDING

Lilija DEGOLA, Elita APLOCIŃA 1474

IMPROVING REPRODUCTIVE PERFORMANCES IN PRIMIPAROUS AND MULTIPAROUS COWS

Tarik MUTEVELIĆ, Edin RAMIĆ, Benjamin ČENGIĆ, Nazif VARATANOVIĆ, Lejla VELIĆ, Amel ĆUTUK 1480

EFFECT OF DIFFERENT NITROGEN FERTILIZER APPLICATION LEVELS ON YIELD AND QUALITY OF THE GREEN FODDER *TRICHANTHERA GIGANTEA*

Tu Quang HIEN, Tu Trung KIEN, Mai Anh KHOA, Tran Thi HOAN, Tu Quang TRUNG 1485

IMPACT OF TEMPERATURE-HUMIDITY INDEX (THI) ON THE EGG WEIGHT OF HYBRID „HISEX BROWN“ LAYING HENS

Bratislav PEŠIĆ, Nebojša ZLATKOVIĆ, Nikola STOLIĆ, Ivana ZLATKOVIĆ 1491

EVALUATION OF LACTOSE, MILK PRODUCTION, SOMATIC CELLS COUNT AND CALVING INTERVAL IN SELECTED FARMING CONDITIONS OF THE SLOVAK SPOTTED DAIRY COWS

Jozef BUJKO, Juraj CANDRÁK, Peter STRAPÁK, Július ŽITNÝ, Cyril HRNČÁR 1498

EFFECT OF SEX ON GROWTH OF ABERDEEN ANGUS CALVES

Radek FILIPCIK, Jan KUČTIK, Vojtech PESAN, Zuzana RECKOVA, Martin HOSEK, Katarina SOUSKOVA 1503

EFFECT OF DIFFERENT DIETARY PROTEIN CONTENT ON PRODUCTION PARAMETERS OF CROSS-BRED FATTENING LAMBS

Aida KAVAZOVIĆ, Almira SOFTIĆ, Teufik GOLETIĆ, Amina HRKOVIĆ-POROBIJA, Dinajda TAHIROVIĆ, Aida KUSTURA, Dragana RUŽIĆ-MUSLIĆ, Kenan PODŽO 1509

NATURAL RADIONUCLIDES IN POULTRY FEED AND ASSESSMENT OF RADIATION RISK

Aleksandra ANGJELESKA, Elizabeta DIMITRIESKA-STOJKOVIK, Radmila CRCEVA-NIKOLOVSKA, Zehra HAJRULAI-MUSLIU, Biljana S. DIMZOVSKA-STOJANOVSKA, Gordana ILIEVSKA.....	1514
COMPARISON OF THE VEAL QUALITY AND CARCASS VALUE UNDER TWO FEEDING CONCEPTS	
Klára VAVRIŠÍNOVÁ, Katarína HOZÁKOVÁ, Ondřej BUČKO, Jana TKÁČOVÁ, Peter JUHÁS, Anna TRAKOVICKÁ.....	1519
NITROGEN USE EFFICIENCY IN DAIRY CATTLE	
Bojan STOJANOVIĆ, Goran GRUBIĆ, Nenad ĐORĐEVIĆ, Aleksa BOŽIČKOVIĆ, Vesna DAVIDOVIĆ.....	1526
EFFECT OF BLUE LUPINE ON THE GROWTH RATE, CARCASS AND MEAT QUALITY OF HEAVY-TYPE TURKEYS	
Robertas JUODKA, Remigijus JUSKA, Raimondas LEIKUS, Rasa NAINIENE, Violeta JUSKIENE	1532
THE EFFECT OF AGE OF PARENT YOUNG BROILER ON THE EFFICIENCY OF INCUBATION OF CHICKENS	
Tatjana ZDRALIC, Sreten MITROVIC, Darko VUJICIC, Milena MILOJEVIC	1540
CHARACTERISTICS OF LOW FAT YOGURT	
Snezana JOVANOVIC, Tanja VUCIC, Marija JEFTIC	1547
RELATIONSHIPS BETWEEN SOMATIC CELL COUNT AND SELECTED QUALITATIVE PARAMETERS OF SHEEP MILK	
Jan KUČHTÍK, Leona KONEČNÁ, Vladimír SÝKORA, Martin FAJMAN, Květoslava ŠUSTOVÁ, Radek FILIPČÍK.....	1553
LIVESTOCK, SUSTAINABLE FOOD SYSTEMS AND THE SUSTAINABLE DEVELOPMENT GOALS	
Víctor SERRA PRIETO, Hamid EL BILALI, Giovanni OTTOMANO PALMISANO, Francesco BOTTALICO, Gianluigi CARDONE, Roberto CAPONE	1559
EFFECT OF SPERM STORAGE TIME AND TEMPERATURE ON SEMEN QUALITY IN THE RABBIT LINE ITELV 2006	
Elhassen LANKRI, Khadideja BOUDOUR, Ahmed AICHOUNI, Fatima RECHACHOU, Nacira ZERROUKI.....	1567
CHARACTERISTICS OF BASIC BEHAVIOURAL FEATURES OF 'HORNLESS HEREFORD' LACTATING COWS RAISED ON A NATURAL GRASSLAND	
Nikolay MARKOV.....	1576
EFFECT OF SALINE WATER ON WATER INTAKE, GROWTH PERFORMANCE AND BLOOD METABOLITES OF BARBARINE LAMB	
Imtiaz BOUZARRAA, Sourour ABIDI, Hichem BEN SALEM.....	1582
EFFICIENCY OF BEANS AND PEAS USED IN THE DIET OF DAIRY COWS	
Aiga TRUPA, Baiba OSMANE	1587
FIRST RECORDS OF <i>ICHTIOPHTHIRIUS MULTIFILIIS</i> ON CYPRINID FISHES FROM AQUACULTURE FACILITIES IN MACEDONIA	
Dijana BLAZHEKOVIKJ - DIMOVSKA, Stojmir STOJANOVSKI	1593
THE INFLUENCE OF FORAGER BEES NUTRITION WITH DIFFERENT POLLENS ON THE DEVELOPMENT OF HYPOPHARINGEAL GLANDS	
Goran JEVTIĆ, Bojan ANĐELKOVIĆ, Snežana ANĐELKOVIĆ, Snežana BABIĆ, Dragoslav ĐOKIĆ, Kazimir MATOVIĆ, Nebojša NEDIĆ.....	1600

RESULTS ON CHROMIUM (Cr) CONCENTRATION IN WILD FISH SQUALIS CEPHALUS & BARBUS BARBUS TISSUES IN VARDAR RIVER, NORTH MACEDONIA	
Lulzim SHAQIRI, Jani MAVROMATI, Vesna ZAJKOVA	1605
GENOMIC DIVERSITY AND LEVEL OF ADMIXTURE IN THE SLOVAK SPOTTED CATTLE	
Kristína LEHOČKÁ, Radovan KASARDA, Anna TRAKOVICKÁ, Ondrej KADLEČÍK, Nina MORAVČÍKOVÁ	1610
RELATION BETWEEN THE FREE SALIVA TESTOSTERONE LEVELS AND BEHAVIOR IN NEW ROOM IN THE PIGLETS UNDER STRESS	
Peter JUHÁS, Juraj PETRÁK, Andrea LEHOTAYOVÁ, Klára VAVRIŠINOVÁ, Katarína HOZÁKOVÁ, Ondrej DEBRECÉNI	1616
6. RURAL DEVELOPMENT AND AGRO-ECONOMY.....	1623
CROP PRODUCTION AND FOOD CONSUMPTION FOR FARMERS' WELFARE IN RWANDA	
Aristide MANIRIHO, Edouard MUSABANGANJI, Philippe LEBAILLY	1624
MONITORING AND EVALUATION SYSTEM IN AGRICULTURAL ADVISORY SERVICE	
Gordana ROKVIĆ, Danijela ČIČIĆ ALJETIĆ	1632
THE SYMBIOSIS OF MODERN INFORMATION AND COMMUNICATION TECHNOLOGIES AND MARKETING STRATEGIES IN AGRICULTURE	
Mirjana MAKSIMOVIĆ, Zvezdana GAVRILOVIĆ	1639
MOBILE AND STATIONARY BEEKEEPING – SUCCESSFUL EXAMPLES	
Vesna OČIĆ, Mislav CAR, Zoran GRGIĆ, Dragan BUBALO, Branka ŠAKIĆ BOBIĆ	1645
THE ROLE OF AGRICULTURAL EXTENSION TOWARDS FACING CLIMATE CHANGE IN AI-GHARBIA GOVERNORATE, EGYPT	
Esam Mohamed EL-BAALY, Manal Mohamed EL-KHADRAGY	1651
RURAL WOMEN NEEDS ABOUT EXTENSION KNOWLEDGE REGARDING CLIMATE CHANGE PHENOMENON IN AI-GHARBIA GOVERNORATE, EGYPT	
Esam Mohamed EL-BAALY, Fatma Mostafa SAAD	1657
ATTITUDE OF AGRICULTURAL EXTENSION AGENTS ABOUT ELECTRONIC AGRICULTURAL EXTENSION IN AI-GHARBIA GOVERNORATE, EGYPT	
Esam Mohamed EL-BAALY	1664
RURAL TOURISM AND SUSTAINABLE DEVELOPMENT : THE CASE OF TUNIS VILLAGE'S HANDICRAFTS, EGYPT"	
Hassan REFAAT, Mohamed MOHAMED	1673
THE ROLE OF INNOVATION SUPPORT SERVICE PROVIDERS IN AGRICULTURAL INNOVATION MANAGEMENT	
Hannu E. S. HAAPALA	1682
WHY INVEST ON INNOVATIVE PRODUCTION? A QUALITATIVE EVALUATION OF THE EMERGING AVOCADO SECTOR IN CRETE, GREECE	
Athanasios RAGKOS, Georgia PAPOUTSI, Magdanili BARDOUNIOTI	1689

IMPLEMENTATION OF THE SUSTAINABLE DEVELOPMENT GOALS IN THE MEDITERRANEAN: INSTITUTIONAL AND GOVERNANCE ARRANGEMENTS Hamid EL BILALI, Gianluigi CARDONE, Giovanni OTTOMANO PALMISANO, Francesco BOTTALICO, Roberto CAPONE	1695
CONTRIBUTION OF GRASSROOTS INITIATIVES TO SUSTAINABLE URBAN FOOD SYSTEMS: THE CASE OF A CAMPUS GARDEN IN MUENSTER, GERMANY Janina WITTENBERG, Hamid EL BILALI, Carola STRASSNER	1703
AGRIBUSINESSES AND RURAL DEVELOPMENT: STATISTICAL EVIDENCES OF POTENTIALS TO GROW NIGERIAN ECONOMY Cornelius Michael EKENTA, Eugenia Chika AKPA, Odekina Menyaga UMAR.....	1710
CONSUMER BEHAVIOUR AT THE LOCAL FOOD MARKET Marta CZEKAJ, Dawid BEDLA.....	1716
ECONOMIC ASPECTS OF HAZELNUT PRODUCTION IN REPUBLIC OF SERBIA Aleksandar MILJATOVIĆ, Veljko VUKOJE	1722
COMPARATIVE ANALYSIS OF ONION PRICES Nebojša NOVKOVIĆ, Ljiljana DRINIĆ, Beba MUTAVDŽIĆ, Otilija SEDLAK, Zorica ZDRAVKOVIĆ.....	1728
FARM PRODUCTION EFFICIENCY IN SERBIA Rade POPOVIC, Zana JURJEVIC, Danilo DJOKIC, Mirjana BOJCEVSKI	1734
THE OPERATIVE PROFIT MARGIN IN RETAIL FOOD Radojko LUKIC, Srdjan LALIC	1740
CROP PRODUCTION IN THE COUNTRIES OF THE WESTERN BALKANS Tihomir ZORANOVIĆ, Vanja ERCEG	1749
STRATEGIC APPROACH TO LAND CONSOLIDATION Milan TRIFKOVIĆ, Žarko NESTOROVIĆ	1755
AGRICULTURAL TRAINING CHALLENGES FACED BY SMALLHOLDER FARMERS IN THE AMATHOLE DISTRICT, EASTERN CAPE IN SOUTH AFRICA Phokele MAPONYA, Sonja Louise VENTER, Christiaan Philippus DU PLOOY, Michael Wolday BAIRU, Erika VAN DEN HEEVER	1761
SOCIAL INNOVATIONS IN MOUNTAIN REGIONS Manfred PERLIK	1771
THE ECONOMIC EFFICIENCY OF THRIFT AND CREDIT COOPERATIVES IN NORTHEAST OF THAILAND Anucha WITTAYAKORN-PURIPUNPINYOO	1776
ROLE OF IMPROVED PRODUCTION TECHNOLOGIES IN ENHANCING FARMERS PRODUCTIVITY IN OILSEED BRASSICAS IN INDO-GANGETIC PLAINS OF INDIA Kartikeya SRIVASTAVA.....	1782
THE WINE ROUTES IN SICILY AS A TOOL FOR RURAL DEVELOPMENT: AN EXPLORATORY ANALYSIS Marzia INGRASSIA, Luca, ALTAMORE, Pietro COLUMBA, Simona BACARELLA, Stefania CHIRONI.....	1788

MULTIFUNCTIONALITY, A STRATEGIC PILLAR FOR AGRICULTURE IN DEVELOPING COUNTRIES: AN EVIDENCE FROM NEPAL	
Kumar BHATTA, Adriano CIANI.....	1796
THE ECONOMIC AND SOCIAL EFFECTS OF LIGHTING DEVELOPMENT IN THE FIELD OF FISHING ON THE PRODUCTIVITY OF FISH WEALTH IN THE GAZA STRIP	
Doaa HUSSEIN, Maria LUCCHETTI	1803
FINANCING NON-AGRICULTURAL ACTIVITIES IN THE RURAL REGIONS OF ROMANIA: A REAL SOLUTION FOR RURAL DEVELOPMENT AT THE BEGINNING OF THE SECOND DECADE OF EU MEMBERSHIP?	
Aurelia - Ioana CHEREJI, Ioan Jr. CHEREJI, Daniela ȚUȚUI	1812
COW MILK ROUTE FROM ROMANIAN DAIRY FARMS TO MARKET	
Irina - Adriana CHIURCIU, Elena SOARE.....	1818
LIVELIHOOD DIVERSIFICATION IN THE CONTEXT OF CLIMATE CHANGE: EVIDENCE FROM FARM HOUSEHOLDS IN SOUTH-EAST TUNISIA	
Fatma ARIBI, Mondher FETOUI, Mongi SGHAIER.....	1824
AN ANALYSIS OF THE CURRENT SITUATION OF SEED SECTOR IN TURKEY	
Nilgün GÜMÜŞAY	1831
SALES MANAGEMENT IN THE DIGITAL ENVIRONMENT	
Rade STANKIC, Niko SADRIC, Natasa PEROVIC	1838
TRADE IN TERMS OF GREEN ECONOMICS	
Svetlana SOKOLOV MLADENović, Đorđe ĆUZOVIC	1843
THE OPERATIVE PROFIT MARGIN IN RETAIL FOOD	
Radojko LUKIC, Srdjan LALIC, Dalibor DONCIC	1849
THE NATIONAL RURAL INSURANCE SYSTEM IN MEXICO: PRIVATE PUBLIC PARTNERSHIP FOR RISK MANAGEMENT	
J. Reyes ALTAMIRANO-CÁRDENAS, Raquel SOLANO-ALONSO, Tania A. VALDEZ-REYES	1858
7. FORESTRY AND AGRO-FORESTRY	1864
FLAMMABILITY OF CERTAIN CONIFERS AND OAK SPECIES OF NORTH WEST ALGERIA	
Yacine BENHALIMA, Dehane BELKHEIR.....	1865
ANALYSIS OF ROAD NETWORK DENSITY AND MEAN SKIDDING DISTANCE IN FOREST MANAGEMENT UNIT "MEŠTREVAC" USING MODERN TECHNIQUES AND METHODS	
Boban MILETIĆ, Dušan STOJNIĆ, Branislav DRAŠKOVIĆ, Anatoly VASILYEVICH GRYAZKIN, Marko GUTALJ, Stefan BOJIĆ	1875
BIOINDICATION OF VITALITY ON THE BASIS OF DIAMETER INCREMENT OF EUROPEAN SILVER FIR (<i>Abies alba</i> Mill.) ON THE MOUNTAIN BORJA	
Goran JOVIĆ, Vojislav DUKIĆ, Branko STAJIĆ, Danijela PETROVIĆ	1884
MONITORING ON APPEARANCE AND SPREAD OF HARMFUL INVASIVE PATHOGENS AND PESTS IN BELASITSA MOUNTAIN	
Margarita GEORGIEVA, Georgi GEORGIEV, Plamen MIRCHEV, Eva FILIPOVA	1890

HEALTH STATUS OF PINE STANDS AS A PART OF THE EXTENSIVE TREE HEALTH MONITORING NETWORK IN BULGARIA

Margarita GEORGIEVA, Plamen MIRCHEV, Georgi GEORGIEV, Gergana ZAEMDZHIKOVA, Maria MATOVA, Eva FILIPOVA, Peter PETKOV 1896

INVESTIGATING FACTORS AFFECTING CONSUMPTION OF CONSTRUCTION TIMBER FOR TEN SELECTED EUROPEAN COUNTRIES. A FOOTPRINT APPROACH

Panagiotis KOYLELIS 1904

CHARACTERIZATION OF *POPULUS* HYBRIDS BY THEIR DIFFERENT RESPONSES TO ABSCISIC ACID DURING *IN VITRO* CULTURE

Greta STRIGANA VIČIŪTĖ, Jonas ŽIAUKA, Tomas JATKAUSKAS, Iwona SZYB-BOROWSKA, Marzena NIEMCZYK, Sigutė KUUSIENĖ..... 1914

BENEFITS BROUGHT BY THE ABUNDANCE AND IMPORTANCE OF FOREST FRUITS FROM SATU MARE COUNTY, ROMANIA

Ciprian TUDOR, Cristinel CONSTANDACHE, Lucian DINCĂ 1920

APPLICATION OF MULTISPECTRAL SENSOR AND SMALL UNMANNED AERIAL SYSTEMS FOR EARLY DETECTION OF STRESS IN FOREST STANDS OF WESTERN SERBIA

Nenad ŠURJANAC, Mara TABAKOVIĆ-TOŠIĆ, Marija MILOSAVLJEVIĆ, Filip JOVANOVIĆ 1926

PARASITOID COMPLEX OF *BRUCHIDIUS TERRENUS* IN BIOCONTROL OF FABACEAE HOST PLANTS AND ITS SPERMATOPHYTES ENTOMOFAUNA

Renata GAGIĆ-SERDAR, Ivana ŽIVANOVIĆ, Tomislav STEFANOVIĆ, Branka SPASOJEVIĆ, Natalija MOMIROVIĆ, Zoran PODUŠKA, Ljubinko RAKONJAC.. 1933

EFFECT OF DROUGHT ON GROWTH AND MULTIPLICATION OF WHITE POPLAR GENOTYPES IN TISSUE CULTURE

Vanja VUKSANOVIĆ, Branislav KOVAČEVIĆ, Saša ORLOVIĆ, Marko KEBERT, Marina KATANIĆ, Lazar PAVLOVIĆ..... 1939

CURRENT STATUS AND POTENTIAL OF SOME IMPORTANT SPECIES OF MANGROVE FOREST IN KIEN GIANG AND CA MAU PROVINCES, VIET NAM

Luom Thanh THAI, Ngon Trong TRUONG 1944

CHARACTERISTICS OF ALKALIZED SOILS IN THE BAČKA REGION (SERBIA) AND THE POSSIBILITY OF THEIR AFFORESTATION

Saša PEKEČ, Marina KATANIĆ, Vladislava GALOVIĆ, Velisav KARAKLIĆ..... 1951

THE EFFECTS OF CLIMATE CHARACTERISTICS ON THE DIAMETER INCREMENT OF DOUGLAS-FIR IN THE CITY OF BELGRADE (SERBIA)

Tatjana RATKNIC, Mihailo RATKNIC, Zoran PODUSKA, Ivana ZIVANOVIC, Nemanja LAZAREVIC, Sabahudin HADROVIC 1956

THE CHOICE OF OPTIMAL TECHNOLOGY OF PROCESSING OF WOOD GREENERY IN THE CONDITIONS OF INTEGRATED UTILIZATION OF FOREST DENDROMASS

Srđan LJUBOJEVIĆ, Ladislav VASILIŠIN, Goran VUČIĆ 1962

ECONOMIC ASSESSMENT OF THE WATER ECOSYSTEM SERVICE PROVIDED BY FOREST AREAS WITH WATER PROTECTION FUNCTIONS IN NORTHWESTERN BULGARIA

Nevena SHULEVA, Paula MALKOVSKA.....	1970
PLANNING THE SIZE OF SAMPLE PLOTS IN THE RESEARCH MACEDONIAN PINE STANDS IN THE STRICT NATURE RESERVATE "MALI BELEG" IN SOUTH SERBIA	
Aleksandar POPOVIĆ	1977
VARIABILITY OF CONCENTRATION OF PHOTOSYNTHETIC PIGMENTS IN LEAVES OF SYCAMORE MAPLE (<i>Acer pseudoplatanus</i> L.) ONE-YEAR-OLD SEEDLINGS OF DIFFERENT HALF-SIB LINES	
Vladan POPOVIĆ, Ljubinko RAKONJAC, Aleksandar LUČIĆ	1985
ANALYSIS OF YIELD QUALITY OF BALD CYPRESS (<i>Taxodium distichum</i> L. Rich.) AT THE LEVEL OF SEED STAND RS-2-2-tdi-00-240	
Vladan POPOVIĆ, Ljubinko RAKONJAC, Aleksandar LUČIĆ	1991
VASCULAR PLANT DIVERSITY AS A VALUABLE TOOL FOR THE ECOLOGICAL AND CULTURAL PROJECTION OF THE NATIONAL PARKS IN GREECE	
Alexandra D. SOLOMOU, George KARETSOS, Panayiotis TRIGAS, Nikolaos PROUTSOS, Evangelia V. AVRAMIDOU, Evangelia KORAKAKI, Konstantinos KOUGIOUMOUTZIS, Konstantinia TSAGKARI	1998
RESTORATION, MANAGEMENT AND VALORISATION OF PRIORITY HABITATS OF MEDITERRANEAN COASTAL AREAS	
Alexandra D. SOLOMOU, Christos GEORGIADIS, Vito E. CAMBRIA, George KARETSOS, Evangelia KORAKAKI, Panagiotis KOULELIS, Evangelia D. AVRAMIDOU, Konstantinia TSAGARI, Panagiotis MICHPOULOS, Luca SCARNATI, Fabio ATTORRE, Efterpi PATETSINI	2004
AUTHOR INDEX	2010

FUNGAL PATHOGENS OF BIRDSFOOT TREFOIL (*Lotus corniculatus* L.) IN SERBIA

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Abstract

Birdsfoot trefoil (*Lotus corniculatus* L.) is a species adapted to field conditions in the most important livestock production region in the all continents and constitutes a very valuable forage species. It has a high ability to utilize nutrients and has very modest demands on the conditions of its growth. The roots of birdsfoot trefoil are associated with bacteria that fix atmospheric nitrogen and, thusly, its populations increase the availability of nitrogen in the soil. This species often forms dense, fibrous root networks that reduce soil erosion. Isolation of the pathogen was done from the leaves and roots. Black leaf spots were observed on birdsfoot trefoil in field plots in Serbia. These spots were circular to irregular. Single lesions often coalesced to form larger lesions and became dark brown. On the roots systems of a large number of plants birdsfoot trefoil symptoms of light to dark brown necrosis and discoloration of conductive tissues were observed. There has not been a systematic research of birdsfoot trefoil mycoflora in Serbia. This research aims to present the results of preliminary research of mycopopulation of 12 different genotypes of birdsfoot trefoil. Total of 480 plant parts have been examined and 7 genera of fungi were isolated: *Alternaria*, *Fusarium*, *Phytophthora*, *Mucor*, *Sclerotinia*, *Bipolaris* and *Rhizoctonia*. Considering the importance of birdsfoot trefoil as a fodder crop in Serbia, the aim of this study was to identify phytopathogenic fungi as casual agents of diseases in birdsfoot trefoil for a clearer perception of problems (the extinction of plants, reducing yields, deterioration of the quality of feed and others) arising as a result of the presence of those fungi.

Key words: *birdsfoot trefoil, fungi, mycoflora.*

Introduction

Birdsfoot trefoil is a widespread plant throughout the world. It originates from Western Europe and North Africa. Birdsfoot trefoil is a very valuable fodder crop that has an important place in our country. It can be cultivated as a single crop or mixed with other crops and used for grazing or for conservation in the form of hay or silage. In addition to being cultivated as a single crop, birdsfoot trefoil has the most important place in grass-leguminous mixtures in the establishment of planted grasslands (Vučković et al., 2010). Birdsfoot trefoil is a plant that thrives on all types of soil, whether sour, lime, sandy, clay or salty. It grows on low quality soils that are not favorable for alfalfa (Vučković et al., 2010).

There are small requirements for soil acidity (Petrović et al., 1996). Birdsfoot trefoil hay contains an average of 18% of crude protein. Birdsfoot trefoil does not cause bloat in ruminant animals (Vučković et al., 2010). The Birdsfoot trefoil is distinguished by its high digestibility and ability to utilize nutrients and very modest requirements considering the conditions of its growth, especially the soil (Petrović et al., 1996). Due to the developed symbiotic bacteria at the root, significant quantities of nitrogen can be fixed annually, which according to some authors exceed 200 kg/ha (Vučković et al., 2010).

Phytopathogenic causative agents of birdsfoot trefoil disease can be present year after year. They are of different intensity and can cause significant losses in yield and the quality of hay. Diseases can limit persistence of *Lotus* spp. in production systems. Several pathogens are involved in a "diseases complex". Nevertheless, little is known about diseases and their impacts, both in the world and ours. Fungal pathogens are the most prevalent organisms and according to the plant tissue they can affect, diseases are classified as follows.

The genus *Epicoccum* was reported in Argentina as a seed pathogen in *Lotus* spp. (Sisterna and Lori, 2005). Also, *Epicoccum nigrum* in Argentina was cited as a causative agent of brown leaf spot of birdsfoot trefoil (Colavolpe et al., 2018).

Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *loti*) is a serious disease of birdsfoot trefoil, reducing yield of forage and seed (Miller-Garvin et al., 2011). Similarly, inducers of diseases such as *Rhizoctonia solani*, *Cercospora medicaginis*, *Sclerotinia trifoliorum*, *Stemphylium botryosum*, *Verticillium albo-atrum*, as well as species of genus *Phytium*, *Leptosphaerulina*, *Phoma*, *Fusarium* and *Alternaria*, are significant disease agents in birdsfoot trefoil as a fodder crop, spread in all the areas of its production (Villegas-Fernández and Rubiales, 2011; Sillero et al., 2014, Vasić, 2015, Vasić, 2017). If care is not taken, the disease can cause serious damage to birdsfoot trefoil seedlings.

Considering the importance of birdsfoot trefoil as a fodder crop in Serbia, the aim of this study was to identify phytopathogenic fungi as casual agents of diseases in birdsfoot trefoil for a clearer perception of problems (the extinction of plants, reduction of yields, deterioration of the quality of feed and other) arising as a result of the presence of those fungi.

Material and methods

For the mycopopulations study, samples were collected from the experimental plant genotypes of birdsfoot trefoil (*Lotus corniculatus* L.) originating from Serbia, from the Rasina region (Gaglovo 1 and 2, Globoder 1, 2, 3, 4, 5, 6 and 7), Mačvanski region (Svileuva 1 and 2) and Pomoravski region (Gložane). The samples were collected between Mart and June 2016-2017 at the location of the Institute for forage crops in Globoder. Parts of plants are carefully washed under running water. After washing, the parts of stem and roots are cut to piece of 0.5-1 cm in size. Prepared samples of roots and stems were disinfected with 96% ethanol for 10 seconds and with 1% sodium hypochlorite (NaOCl) for 1 minute and then washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (PDA) with streptomycin. Five pieces of the plant parts (roots and tree) were placed in each Petri dish in four replications. They were kept in a thermostat at 25°C in 12 h light / 12 h night regime. The observations were performed every 3 days, and the majority of mycelium samples were developed up to 14 days. Developed mycelia were screened to a new PDA substrate and, after an initial grow, the peak part of the mycelium was reseeded on PDA again.

Microscopic examination was performed using microscopes Olympus CX31. Morphological identification of fungi to the genus was carried out using a standard key. Calculated by the frequency of isolation in % according to the formula Vrandečić *et al.* (2011):

$$(\%) \text{ Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results and discussion

In the study of mycopopulations of birdsfoot trefoil genotypes, total of 480 plant parts were analyzed. Fungi were isolated on all plants from birdsfoot trefoil, and there were clear symptoms on stems in the form of spots and necrotic lesions. Fungi from genus *Alternaria*, *Phytophthora*, *Fusarium* and *Rhizoctonia* were isolated from these plants. Also, in small number of plants, there were necroses with white airy mycelium in the lower third of stems and fungi from the genus *Sclerotinia* and *Bipolaris* were isolated from those plants. Also, saprophytic fungi from the genus *Mucor* were isolated (Table 1).

Table 1. Frequency of fungal isolation on *Lotus corniculatus* L.

Genotypes	Number of samples		Fungi species - stem	(%) Isolation frequency	Fungi species - root	(%) Isolation frequency
	Plant part - leaf	Plant part - root				
Globoder 1	20	20	<i>Alternaria</i> sp. <i>Fusarium</i> sp. <i>Phytophthora</i> sp.	15 10 25	<i>Rhizoctonia</i> sp. <i>Fusarium</i> sp.	40 35
Globoder 2	20	20	<i>Alternaria</i> sp.	60	<i>Rhizoctonia</i> sp.	75
Globoder 3	20	20	<i>Phytophthora</i> sp. <i>Alternaria</i> sp. <i>Mucor</i> sp.	25 20 40	<i>Fusarium</i> sp.	85
Globoder 4	20	20	<i>Alternaria</i> sp.	35	<i>Rhizoctonia</i> sp.	65
Globoder 5	20	20	<i>Fusarium</i> sp. <i>Rhizoctonia</i> sp.	25 10	<i>Fusarium</i> sp. <i>Rhizoctonia</i> sp.	65 20
Globoder 6	20	20	<i>Phytophthora</i> sp.	60	<i>Fusarium</i> sp. <i>Rhizoctonia</i> sp.	10 85
Globoder 7	20	20	<i>Alternaria</i> sp. <i>Mucor</i> sp.	40 15	<i>Rhizoctonia</i> sp.	75
Svileuva 1	20	20	<i>Rhizoctonia</i> sp.	35	<i>Fusarium</i> sp. <i>Rhizoctonia</i> sp.	25 70
Svileuva 2	20	20	<i>Fusarium</i> sp.	10	<i>Fusarium</i> sp. <i>Rhizoctonia</i> sp.	20 65
Gaglovo 1	20	20	<i>Alternaria</i> sp.	60	<i>Fusarium</i> sp.	75
Gaglovo 2	20	20	<i>Sclerotinia</i> sp. <i>Bipolaris</i> sp.	30 35	<i>Fusarium</i> sp.	85
Gložane	20	20	<i>Alternaria</i> sp. <i>Fusarium</i> sp.	15 35	<i>Fusarium</i> sp.	90

The symptoms of a light to dark brown necrosis on the root system of the plants were observed, and from these plants fungi of the genera *Rhizoctonia* were isolated. Discoloration of the conductive tissues of the root system was observed in a large number of plants, and from these plants, fungus of the genus *Fusarium* was isolated (Table 1).

Isolations were conducted in all the birdsfoot trefoil plants with clearly visible symptoms of the disease.

In these studies, there was a difference in the frequency of isolation of certain genera of phytopathogenic fungi in birdsfoot trefoil genotypes originated from different regions of Serbia. It has been observed that in birdsfoot trefoil genotypes that originated from the Rasina region, fungi of the genera *Alternaria*, *Rhizoctonia* and *Fusarium* were more frequently

isolated. While the genera *Sclerotinia* and *Bipolaris* were isolated in significant percentages on one sample originating in the Rasina district (Table 1). Likewise, genera *Fusarium* and *Fusarium* were more often isolated from genotypes originated from the Pomoravlje region. While in the Mačva region isolated fungi from the genus *Fusarium* and *Alternaria*.

The results indicate that birdsfoot trefoil is vulnerable to the attack of a large number of phytopathogenic fungi that can have a significant impact on reducing its yield and quality.

In all the plants, in which isolations were conducted, there were clearly visible symptoms of the disease present. In these studies, there was difference in frequency of isolation of some genera of phytopathogenic fungi in birdsfoot trefoil genotypes from three regions in Serbia. It was observed that in the genotypes that originated in Serbia, fungi of genus *Rhizoctonia*, *Fusarium*, *Alternaria*, *Phytophthora* and *Sclerotinia* were frequently isolated.

Birdsfoot trefoil are adversely affected by numerous fungal diseases leading to a steady reduction in the cultivated area in many countries. Crown and root diseases considered chronic diseases, causing plant death and losses of 60 to 80%. Typical symptoms are rot and wilt. The main Genus is *Fusarium* (*F. solani*, *F. oxysporum*, *F. verticillioides* and *F. equiset*) (Sisterna and Lori, 2005). Stem and foliar diseases onse do not cause directly the plant death. They contribute to the progressive weakness of the plant through the effects on the basic metabolic processes. The reported pathogens are *Phomopsis loti* (blight) *Colletotrichum destructivum* (anthracnose), *Stemphylium loti* (leaf spot) (Vasić et al., 2016, Sisterna and Lori, 2005). Seed diseases they cause decrease in germination and damping off. Fungi genera present in the seed are: *Alternaria*, *Aspergillus*, *Bipolaris*, *Botrytis*, *Cladosporium*, *Colletotrichum*, *Culvaria*, *Epicoccum*, *Fusarium*, *Leptosphaearulina*, *Penicillium*, *Phoma*, *Phomopsis*, *Stemphylium* and *Verticillium* (Sisterna and Lori, 2005).

The *Fusarium oxysporum* isolates pathogenic to birdsfoot trefoil have a unique host range relative to other pathogenic *Fusarium oxysporum* tested; they cause severe vascular wilt on birdsfoot trefoil but not on alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), dry bean (*Phaseolus vulgaris* L.), or soybean [*Glycine max* (L.) Merr.]. *Fusarium oxysporum* f. sp. *loti* is only known to occur in USA (Miller-Garvin et al., 2011). Altier et al. (2000) showed that one cycle of phenotypic selection in the greenhouse using a composite of Uruguayan *Fusarium oxysporum* isolates increased the level of resistance to *Fusarium* root and crown rot in birdsfoot trefoil adapted to Uruguay. The Uruguayan *Fusarium oxysporum* isolates cause vascular wilt and root necrosis similar to the disease symptoms caused by *Fusarium oxysporum* f. sp. *loti*, but the wilt symptoms develop more slowly. The Uruguayan isolates of *Fusarium oxysporum* are primarily associated with disease development on mature plants, with disease symptoms and plant mortality becoming most severe in the second and third production years. *Fusarium oxysporum* f. sp. *loti* causes considerable plant mortality at the seedling stage Miller-Garvin et al. (2011).

Al-Jaradi et al. (2018) state the fungus of the genus *Alternaria* spp. causing various types of spots and blights on *Vicia faba*, *Pisum sativum* and *Vigna unguiculata*. Likewise, Coca-Morante and Mamani-Álvarez (2012) detected fungus from the genus *Alternaria* on *Vicia faba* plants in Bolivia. At three sites in Bolivia, *Alternaria* sp. were found during the vegetative growth of the plants. Problems appeared as isolated spots. Colavolpe et al. (2018) in Argentina for the first time stated *Epicoccum nigrum* as causing a brown leaf spot of birdsfoot trefoil. On infected plants birdsfoot trefoil, yellow spot and black spots were spotted (Sisterna and Lori, 2005).

Conclusion

This paper presents the preliminary results of mycopopulations research in 12 experimental birdsfoot trefoil genotypes. Birdsfoot trefoil is an important forage crop and its importance as livestock feed is growing within our country. This research is the beginning of a more

comprehensive study of phytopathogenic fungi on of birdsfoot trefoil. So far, there were no significant researches in this direction in Serbia, so the future researches related to birdsfoot trefoil will go in the direction of selection of genotypes with increased tolerance to fungal diseases.

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