Component I: Systematics and Identification

1c. Microorganisms Associated with Insects and Weeds (U.S. Value 2007: $ 202 billion)

Problem Statement: The full potential of microbes as biological control agents has not been realized because our knowledge of the relationships between microorganisms and other organisms is limited by confusion over a basic understanding of:

- Species and Strain Identification, Discovery, and Description
- Genetic Relationships
- Environmental and Biological Effects on Geographic Distribution and Dispersal
- Fundamental Biology of Microorganisms Associated with Arthropods and Weeds

***Research Needs*:**

1. **Species and Strain Identification, Discovery, and Description**

   Importance: Strains and species of agriculturally important microorganisms must be identified, and newly discovered ones need to be described. New isolates are accessioned into the core ARS national culture collections, and then distributed to ARS scientists, customers, partners and stakeholders. These new microorganisms can lead to innovations in biological control of insects, mites and weeds, improvements to methods for rearing insects (especially those currently difficult or impossible to rear in the laboratory), improvements to agricultural crops and commodities, and advancements in a number of other increasingly important areas of agricultural research (e.g., biofuels).

   Research Gaps: Current definitions of what constitutes a species are inadequate to describe microorganisms so the identity and naming of many species of microorganisms is controversial. Methods are lacking for culturing "uncultivable" microorganisms, which, if manipulated more easily in the laboratory, could possibly be used for agricultural applications.

   Actions: ARS will:

   - Develop new and/or improved analytical methods of data analysis as well as bioinformatic tools that enhance species and strain identification.
   - Refine existing criteria and develop novel ones for the recognition of species and strains from the following groups of microorganisms: insect-associated bacteria; insect-associated fungi (including insect-associated microsporidia);

insect-associated viruses; weed-associated bacteria; weed-associated fungi; and weed-associated viruses.

- Develop novel methods for the growth and culture of fastidious and/or currently "non-cultivable" microorganisms.
- Distribute insect- and weed-associated reference strains to customers, partners and stakeholders via the following: core National Culture Collections; the ARS Culture Collection at Peoria (Illinois), a general culture collection housing over 90,000 type and reference stains (Peoria, Illinois); and the ARS Collection of Entomopathogenic Fungal Cultures at Ithaca (New York), which comprises more than 9,000 type and reference strains.
- Transfer strains of potential or demonstrated agricultural value to the above core National collections.

2. Genetic Relationships

Importance: Rapid diagnostic tests designed to differentiate species and strains of pathogens and their potential impact on agricultural and natural resources are based on their genetic relationships with well-understood species and strains. Scientists or regulators identify target species or strains as well as other closely related species or strains in order to predict properties such as toxigenicity and virulence.

Research Gaps: The number and diversity of species and strains of microorganisms whose genetic relationships are unknown is immense. For example, most genetic relationships among insect- and weed-associated microorganisms have not been demonstrated with modern methods of analysis. This is compounded by the lack of easily identified physical characteristics of microorganisms and the recent discovery that certain genes used extensively to assess the genetic relationships among microorganisms, such as the 16S ribosomal RNA gene of bacteria, are inadequate for comparing all species and strains.

Actions: ARS will:

- Characterize the utility of alternative genes for resolving genetic relationships among species and strains of microorganisms with different levels of relatedness.
- Determine genetic relationships of insect- and weed-associated microorganisms, including (but not limited to): insect-associated bacteria (*Bacillus, Enterobacter, Serratia, Spiroplasma* and *Pseudomonas*); insect-associated fungi (*Beauveria, Candida, Cordyceps, Cryptococcus, Isaria, Metarhizium, Nomuraea, Paecilomyces, Verticillium*), including microsporidia; insect-associated viruses; weed-associated bacteria; weed-associated fungi (*Fusarium*); weed-associated viruses.

- Develop rapid, molecular-based diagnostic tests to detect and differentiate crop pathogens vectored by insects and/or weeds.

3. **Environmental and Biological Effects on Geographic Distribution and Dispersal**

Importance: Study of the environmental and biological processes that shape the geographic distributions of genetic lineages of insect- and weed-associated microorganisms across time has been neglected, but is extremely important for a number of agricultural purposes. This knowledge will help predict future changes in the spread of plant-diseases brought about by climate change. As an example, the end of the last ice age brought about rapid and substantial changes in climate and available habitat due to
retreating glaciers, which in turn allowed for the northward expansion of temperate deciduous forests and warm grasslands. Likewise, today's pattern of global climate change will allow more southerly species of fungal pathogens to expand northward by being carried along with the concomitantly expanding ranges of southerly species of weeds and insect vectors.

Research Gaps: We lack an examination of the population structure within a geographic context of various weed and insect-associated microorganisms, particularly, plant pathogens that have been listed as a high priority for customers, partners and stakeholders. In addition, the time when contemporary populations became genetically isolated is undetermined, and the factors affecting the separation unidentified.

Actions: ARS will:

- Determine how genetic diversity is partitioned within and between populations of insect-associated and weed-associated microorganisms.
- Clarify the historical timeframe for the separation of genetically distinct North American insect-associated and weed-associated populations of microorganisms from populations in other regions of the world and identify the factors that maintain or disrupt this separation.
- Identify the historical environmental and biological processes that facilitate transmission of plant pathogens from weed reservoirs to crops in North America.

4. **Fundamental Biology of Microorganisms Associated with Arthropods and Weeds**

Importance: The toxicity of a microorganism is a product of the chemical interactions between the microorganism and its host. These interactions are key targets for new intervention and implementation strategies for effective weed and arthropod control because production of these chemicals is genetically controlled and can be artificially exploited in a laboratory. This type of study, however, is

only possible with microorganisms for which the genetic code is largely or entirely known.

Research Gaps: Several fungi and bacteria have been commercially registered as biological control agents, but little genetic information from this group of agricultural pathogens is available.

Actions: ARS will:

- Identify the genes and their chemical products driving the association between agriculturally-relevant microorganisms and their host systems.
- Determine specific virulence and pathogenicity factors in microorganisms important for biological control.
- Identify secondary metabolites from microorganisms that may lead to new chemistries for agricultural use.

***Anticipated Products*:**

- Expanded knowledge of the diversity of microorganisms associated with insects and weeds.
- Improved methods for identifying microorganisms associated with insects and weeds.
- Accurate genetic relationships and names of insect- and weed-associated species and strains of microorganisms.
- Novel and enhanced species identification databases for insect- and weed-associated microorganisms.
- Improved knowledge of the environmental and biological factors that influence the geographic dispersal of microorganisms.
- Diagnostic methods providing the ability to rapidly and accurately identify beneficial microorganisms.
- Accurate and confident post-release identification of pathogens approved and released for biological control.
- New reference strains for research and industrial or commercial use.

Potential Benefits (Outcomes):

- Reductions in crop losses from plant diseases transmitted by insect pests of citrus.
- Improved ability to accurately identify insect- and weed-associated microorganisms.
- Improved prediction of biological control potential of new strains.

- Development of molecular diagnostic assays for tracking releases of biological control strains.
- Availability of information to regulatory agencies for risk assessment and approval of petitions for release of biological control strains.
- Ability to culture and preserve "difficult" microorganisms and microorganisms currently classified as "non-cultivable."
- Distribution of microbial strains from core ARS national collections to customers, partners and stakeholders for research and development.

***USDA ARS Resources*:**

- ARS Collection of Entomopathogenic Fungal Cultures (http://arsef.fpsnl.cornell.edu)
- Biological Integrated Pest Management Unit, Ithaca, New York
- Foreign Disease-Weed Science Research Unit, Ft. Detrick, Maryland