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RNAi: Silencing genes for pest management

RNAi process

The previous issue of the *IPM Chronicle* discussed the development of gene editing techniques to enhance crops. The discussion will now turn to a molecular technique that provides the ability to turn off (silence) genes within an organism's cells. This process of silencing genes can be accomplished through a process called ribonucleic acid interference or RNAi.

Developed in the 1990s, RNAi is now being studied as a tool for managing various pest species. So what is RNAi, and how exactly can it be used for pest management? Before these questions can be answered, one must first understand the connection between DNA and proteins.

DNA-RNAi relationship

DNA is the molecule that contains all the information needed to build and maintain an organism. More specifically, genes (sections of a DNA molecule) provide the information necessary to build specific protein molecules that carry out the various functions of a cell. However, a gene does not build a protein directly. The bridge between DNA and protein synthesis is RNA. In the simplest sense, RNA molecules convert the information stored in DNA into proteins.

There are many types of RNA molecules involved in protein synthesis, and each performs a specific function. The role of RNAi is to turn down the production of specific proteins within a cell, or remove foreign RNA molecules that may have been inserted by an invading organism, such as a virus.

Advantages of RNAi

By exploiting the RNAi pathway, scientists can target specific RNA sequences for removal before they can be used to make a protein. Thus, RNAi technology can potentially be used to silence a gene's activity that is essential for the development and survival of particular pest species. There are many potential advantages of using RNAi technology in pest management. The ability to target an individual pest species without harming non-target species in the environment is the most important benefit. In addition, the likelihood of a pest developing resistance would be extremely low, especially if multiple genes are targeted simultaneously. Delivery of RNAi can be conducted in a manner similar to pesticides, such as in a spray or powder. Additionally, RNAi could be delivered to pests through the genetic engineering of transgenic plants, similar to Bt crops.

Current studies

RNAi is currently being studied for control of several important agricultural insect pests including western corn rootworm (*Diabrotica v. virgifera*), Colorado potato beetle (*Leptinotarsa decemlineata*), cotton bollworm (*Helicoverpa armigera*), beet armyworm (*Spodoptera exigua*), green peach aphid (*Myzus persicae*) and silverleaf whitefly (*Bemisia tabaci*). RNAi technology is also being used to develop plant varieties resistant to viruses, such as cucumber and tobacco mosaic virus and tomato spotted wilt virus (among many others), and is being considered to combat herbicide-resistant weeds. In addition to its many potential agricultural uses, RNAi technology is being used to study gene function for the treatment of a variety of human diseases, such as cancer, HIV, hepatitis and malaria.

Milkweed and monarch conservation

With their brilliant orange and black pattern, monarch butterflies (*Danaus plexippus*) are one of the most recognizable butterfly species across North America. Their migration can cover thousands of miles and is one of the most amazing migration stories.

Like most butterflies, the monarch's diet changes as it develops from its larval (caterpillar) to adult (butterfly) stage. While adult monarchs feed on nectar from a wide variety of flowers, including milkweed, monarch caterpillars feed exclusively on milkweed plants in the genus *Asclepias*.

This complete dependence on milkweed makes the monarch particularly sensitive to changes in habitat.



Figure 1. Monarch caterpillars feed exclusively on milkweed plants. (Photo credit: Sheldon Owen)

Seriousness of the problem

The most significant risk the monarch butterfly faces is habitat loss; in North America, this means the loss of milkweed. Experts estimate that in the eastern U.S., monarch populations have declined by 90 percent in the past 20 years. The decline is so significant that monarchs are now being considered for protection under the Endangered Species Act.

To help the monarch, West Virginia is teaming with other states to develop a habitat improvement plan that will ensure the monarch's habitat is sustained at high enough levels to bring back monarch populations.

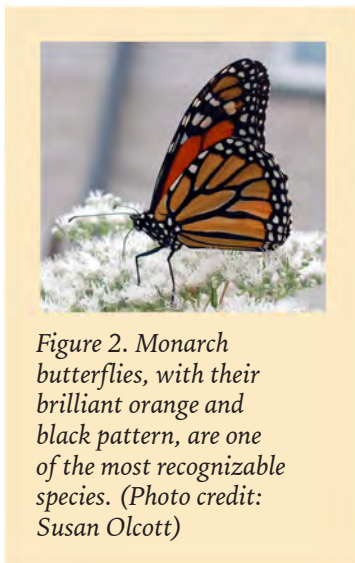


Figure 2. Monarch butterflies, with their brilliant orange and black pattern, are one of the most recognizable species. (Photo credit: Susan Olcott)

How to help

There are three species of native milkweed in West Virginia: common milkweed (*Asclepias syriaca*), swamp milkweed (*Asclepias incarnate*) and butterfly milkweed (*Asclepias tuberosa*).

In some cases, these varieties are viewed as an invasive pest in the pastures of West Virginia. Control efforts are often utilized to rid fields of these plants because milkweed can be potentially toxic to livestock. If quality forage is available, then milkweed typically is not desired by livestock. Also, avoid using hay or prepared forages that contain milkweed or turning hungry livestock into fields that contain milkweed.

West Virginians can help by conserving milkweed patches on their land and planting marginal areas with milkweeds and other native flowers. Even small milkweed habitat areas scattered along the migration route are important to the conservation efforts. This will give monarch caterpillars the food sources they require and provide monarch butterflies with the nectar resources needed to fuel their trans-continental migration. As an added benefit, when you provide monarch habitats, you are also providing a habitat for other pollinators, such as honeybees and native bees.

Milkweed native to West Virginia can be purchased from native plant nurseries and seed companies in both plug and seed form. It can also be propagated at home by collecting and sowing local seeds. It is important to plant native varieties to ensure the best establishment, growth and food source for the native pollinators.

Conservation efforts

The conservation practices commonly used to benefit monarch butterflies and native pollinators include planting cover crops, planting flowering woody and herbaceous vegetation along hedgerows over critical areas and riparian buffers, and fencing livestock out of habitats to allow milkweed and native wildflowers to flourish.

The 2014 Farm Bill retained all of the conservation provisions that make pollinators and their habitat a priority for the United States Department of Agriculture. The Natural Resource Conservation Service administers many conservation programs

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Beware of toxic weedy parsnips

Although they provide contrasting color, size and texture, not all plants that produce large, vivid blooms are desirable in a garden. Certain weeds that belong to the carrot family (Apiaceae) should be avoided because of their toxicity and ability to cause dermatitis. Wild parsnip (*Pastinica sativa*) and cow parsnip (*Heracleum maximum*) are among the weedy parsnips that can be found planted in West Virginia gardens.

Wild parsnip

Wild parsnip is a relative of the cultivated parsnip and can be seen growing in early spring along roadsides, ditches and the perimeter of fields. Wild parsnip is a biennial capable of producing vivid, yellow flowers upon bolting during its second year of growth. They grow in umbels, or flower clusters, that form into a flat or curved surface, and their leaves have saw-toothed edges.

All parts of the plant are toxic to mammals because of secondary metabolites called furocoumarins. Secretions can cause dermatitis upon contact and photodermatitis (rash caused by exposure to sunlight).

Cow parsnip

Cow parsnip is another biennial plant with succulent stems that produce large white umbels during the second year or subsequent years (it may grow as a short-lived perennial). Cow parsnip is not as toxic as the wild parsnip, but it can also cause rashes upon contact with the plant sap.



Figure 3. An umbel of a wild parsnip plant with distinctive yellow flowers during the second year's growth. Wild parsnip can cause dermatitis upon contact with skin. (Photo credit: Greg Hamons)



Figure 4. Growth habit of a wild parsnip characterized by leaves with saw-toothed margins and ridged stems. (Photo credit: Greg Hamons)



Figure 5. Cow parsnip umbel with distinctive white flowers during the second year's growth; plant sap can cause dermatitis upon contact. (Photo credit: Rakesh Chandran)



Figure 6. Growth habit of a cow parsnip characterized by large tri-lobed leaves. (Photo credit: Rakesh Chandran)

Other weedy biennials

Giant hogweed (*Heracleum mantegazzianum*) is a non-native invasive capable of causing severe dermatitis. It is a close relative of weedy parsnip and can sometimes be mistaken for the Angelica plant (*Angelica sylvestris*). Fortunately, giant hogweed is yet to be documented in West Virginia, although it is present in Ohio and Pennsylvania. Giant hogweed produces larger flowers and leaves with deep lobes (as fingers in a palm) as opposed to the three major lobes found in cow parsnip.

Poison hemlock (*Conium maculatum*), water hemlock (*Cicuta virosa*), and wild carrot or Queen Anne's lace (*Daucus carota*) are other weeds with varying degrees of toxicity. These weeds, however, are not typically planted in a garden.

Managing weedy parsnip

The best time to manage weedy biennials in pastures is during the vegetative stage in the first year's growth. Mechanical removal works for managing small populations, while herbicides are more effective in controlling larger infestations.

Apply a systemic herbicide when they have sufficient foliage and are actively growing. Herbicides such as Grazon P+D (picloram + 2,4-D; restricted use pesticide), Weedmaster (2,4-D + dicamba) or Crossbow (triclopyr+2,4-D) are effective options. Contact your WVU Extension Service county agent for questions regarding safe and proper use of pesticides.

Lady beetles rescue pepper plants from aphids

Last fall, a crop of peppers was started in the greenhouse and there was a standing order for biological control agents to be delivered as part of our integrated pest management program. Everything was in place and there should not have been any problems, but then aphids were spotted in the pepper flowers (Figure 7).



Figure 7. WVU undergraduate research student, Chelsie Chapman, releasing lady beetles on aphid-infested pepper plants. (Photo credit: Daniel Payne)

Different options for control were considered. Sprays were quickly eliminated as an option since the peppers were going to be eaten and a spraying into the flowers would be difficult. If a biological control agent could be used, it was crucial to find out which aphid was the culprit. The aphid in question turned out to be the foxglove aphid, which is difficult to control biologically. In addition, we used two parasitoids that did not stop the foxglove aphid's advances. Therefore, the options were limited to releasing the lady beetles.

The convergent lady beetle, *Hippodamia convergens*, is not grown in a facility, but rather caught in the wild in the western U.S. (typically California) and shipped throughout the U.S. for aphid control. They are an inexpensive, generalist predator (a pint of 9,000 was \$50) that prefers aphids, but also consumes scales, thrips and small caterpillars. During their lifetime, one lady beetle can consume 5,000 aphids (Figure 8). One disadvantage is lady beetles do not stay in place without prey to eat.

By the time the lady beetles arrived, the aphid infestation had moved to the rest of the plants. We released half of the lady beetles when they arrived and they immediately colonized the plants. Within a week, our plants were aphid-free. Amazingly, a small number of the lady beetles have stayed in the greenhouse and continued guarding the plants from the aphid invaders (Figures 9 and 10).



Figure 8. Lady beetles on pepper plants. (Photo credit: Daniel Payne)

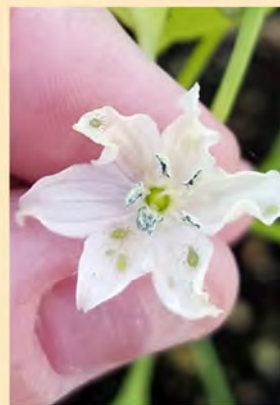


Figure 9. Foxglove aphid in pepper flower. (Photo credit: Barbara Liedl)

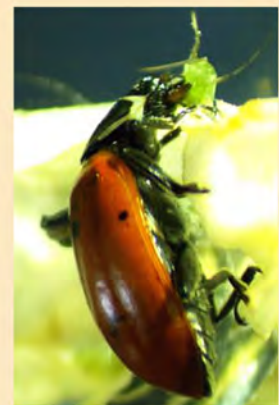


Figure 10. Lady beetle eating a foxglove aphid. (Photo credit: Barbara Liedl)

Milkweed and monarch conservation

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that provide not only technical assistance, but also cost-share opportunities for landowners, to implement conservation practices. Please contact your local NRCS office to find out more about these programs.

For more information on monarch butterfly conservation and milkweeds, contact WVU Extension Specialist Sheldon Owen at 304-293-2990 or sheldon.owen@mail.wvu.edu.

Organic options for strawberry black root rot complex and crown rot management

Strawberry black root rot complex and crown rot are caused by multiple soilborne fungi that can severely affect plant vigor and productivity. If strawberries are planted in contaminated soil, loss in plant vigor becomes evident within a few months of planting and plant mortality rises during harvest – especially under conditions of environmental stress. In a perennial strawberry production system, disease severity increases each year leading to increased yield variability and eventual productivity loss.

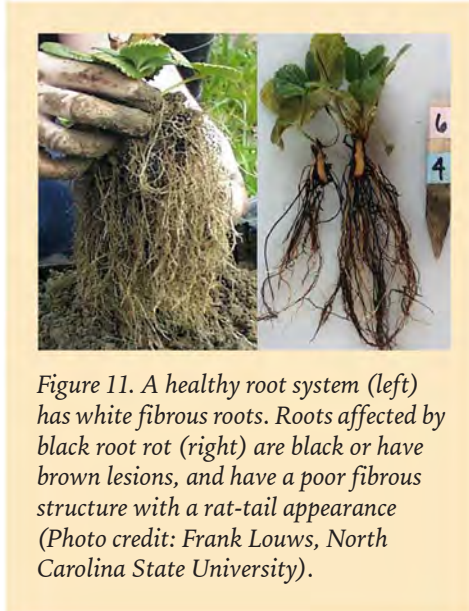


Figure 11. A healthy root system (left) has white fibrous roots. Roots affected by black root rot (right) are black or have brown lesions, and have a poor fibrous structure with a rat-tail appearance (Photo credit: Frank Louws, North Carolina State University).

Causes

Although a few fungal pathogens and nematodes have been associated with black root rot, *Rhizoctonia* spp. and *Pythium* spp. are documented as causal agents. *Colletotrichum gloeosporioides*, *Phytophthora cactorum* and *Fusarium* spp. infections also can quickly move to the crown resulting in crown rot and plant wilting. Lesion nematodes may predispose roots to black root rot-causing fungi that will develop into black cortical lesions. These lesions may girdle the whole root resulting in loss of function and mass giving the root a rat-tail appearance (Figure 11).

For U-pick strawberry production, growers prefer to grow strawberries in suitable plots year after year. As a result, soilborne pathogen populations build up over time causing significant disease problems and yield reduction. This is a much more significant problem for growers who utilize perennial matted row system with limited crop rotation options.

Management

With the phasing out of methyl bromide, soil fumigation techniques involving methyl bromide alternatives (synthetic chemicals) are not as effective and very difficult to apply due to new regulations. It emphasizes the importance of treating/keeping plants infection-free beginning with the nursery-produced planting stock. Options for sustainable management of black root rot complex and crown rot include:

- **Rotation** is the first line of defense. Growers should select a new piece of land for each new planting. The strawberry black root rot problem is similar to apple replant disease in that it manifests itself when susceptible plants are placed into soil previously occupied by a related species.
- **Pre-colonizing** the plant root system with beneficial microbes will boost plant vigor and keep the harmful microbes at bay. Strawberry plug plant producers can treat the tips and planting mix with a commercially available product suspension, such as Serenade, Terragrow, etc., before plug setting. Before planting, fruit growers can dip the plug or the plant's bare root system in product suspension overnight to facilitate root system colonization. Studies indicate that probiotic bacteria-treated strawberry plants have higher vigor and less root disease.
- **Biofumigation** (the use of biologically active plant substances to control soilborne pests and diseases) of plots with high glucosinolate containing mustard cover crop, such as 'Caliente 199' or mustard meal, can provide both microbial suppression, as well as adding organic materials to the soil. If biofumigation cover crop is grown to the optimum stage and tissues are softened then immediately incorporated into the soil, it can suppress soil fungi and nematodes in addition to increasing soil biomass.
- **Anaerobic soil disinfestation** on selected plots can be done in three steps: 1) Incorporate organic material to provide carbon (C) source to activate soil microbes. Options for C source include rice bran at 8 tons/acre, mustard meal at 1 ton/acre or any legume or grass cover crop biomass; 2) These C sources are mixed into the soil with a walk-behind tilling machine, and then the area is covered with oxygen-impermeable tarp; 3) Soil is irrigated to saturation to create anaerobic conditions. This stimulates the anaerobic decomposition of the added organic material and enhances the diffusion of by-products in the soil. Accumulation of toxic/suppressive products deriving from the anaerobic decomposition kill pathogenic microbes. This process takes at least three weeks to complete.



Which is best? Fall planting or spring planting

People often wonder when the best time to plant is – fall or spring. The fact is that both of those seasons are excellent for planting trees, shrubs and herbaceous perennials.

Fall Planting

In areas where an early fall frost is unlikely, fall planting is recommended because the soil is still warm, rains are frequent and being outside is still enjoyable.

Fall planting gives plants a jump-start on the growing season, which results in more robust plant growth. Trees, shrubs and herbaceous perennials have plenty of time to establish their roots, which continue to grow at a slow rate at the low temperatures of 45 and even 42 F. By spring, the transplanted or newly planted plants will have a sufficient root system developed in the fall in a previous season.

Here are a few tips for fall planting:

- Planting should be done about two months, and a minimum of six weeks, before the first average frost date. That gives plants enough time to repair and develop new roots before the ground freezes.
- Fall rains ensure good plant establishment. If there is a dry fall, watering the new transplants is a must. About an inch of water per week will need to be added to keep them well irrigated.
- Mulch preserves soil moisture and facilitates root development. Transplant shock is enhanced by inadequate soil moisture that is either too dry or too wet.
- Mulching also helps prevent rapid cooling of the soil. Moist soil tends to stay warmer longer.
- Fall is an ideal time to divide some of the spring and summer blooming plants. The new plants/divisions will be able to establish themselves enough for a good start in the spring.

Spring Planting

In areas where an early fall frost is likely, spring planting is preferred. Planting should be done after the last spring frost. By that time, the soil temperature should be in the high 40s to mid 50s F. Gardeners, and growers alike, should consider the average date for the last spring frost occurrence in their region. There are interactive maps available at <http://www.plantmaps.com/interactive-west-virginia-last-frost-date-map.php> that will provide that information according to zip code. Typically, the average last spring frost date in southwest West Virginia and parts along the Ohio River and southeast Ohio border is in late April. In the eastern and north central

part of the state, this date is closer to mid-May. In the northeastern, mountainous region of the state, the average last spring frost date is in late May (Figure 12).

Here are a few tips for spring planting:

- At least 10 days prior to planting, remove mulch from the beds or designated planting areas to accelerate soil warm-up.
- Loosen the soil by digging the hole big enough to accommodate roots without having to bunch them up or fold them. Allow plenty of room to spread around to avoid coiling and subsequent girdling. The standing rule is that the hole should be about three times larger than the root system and twice as deep to allow for backfill and positioning of the plant. The plants should be buried at the same depth as they were in the nursery or in the pot.
- Water generously immediately after planting to push out any air pockets around the roots and to ensure a good, firm contact between the roots and the soil.
- Apply mulch 3 to 4 inches deep. Pull the mulch away from the bark at the base of the tree trunk to avoid potential collar rot, winter injury, root rot, and increased insect and rodent damage.

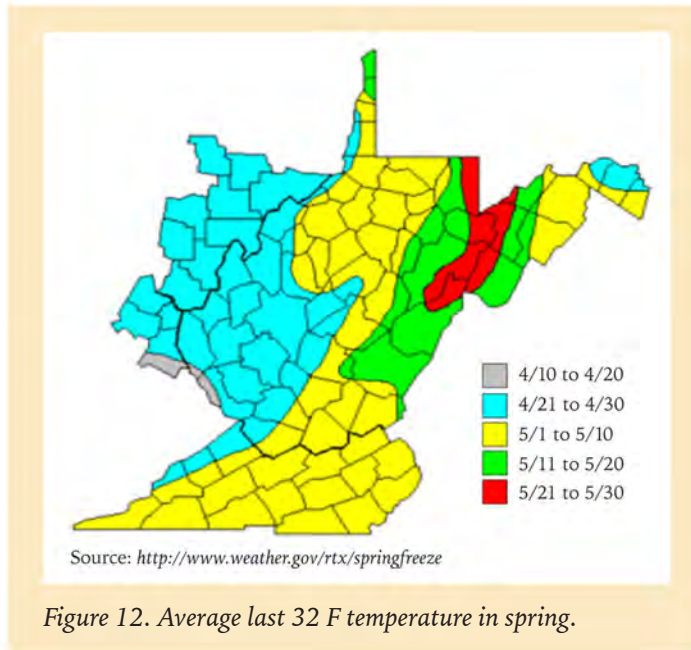


Figure 12. Average last 32 F temperature in spring.

Watch for a new invasive insect pest

West Virginians should be on the lookout for a new invasive insect pest, the spotted lanternfly (*Lycorma delicatula*), which attacks several host plants such as grapes, hops, and various fruit and timber trees. Native to Asia, this pest was first detected in Pennsylvania in the fall of 2014. Even though, the spotted lanternfly is not known to occur in West Virginia, the insect has been spreading and is now confirmed in 13 counties in southeastern Pennsylvania and in New Castle County, Delaware.



Figure 13. Spotted lanternfly adults. (Photo credit: Daniel Frank)

Description

Spotted lanternfly adults (Figure 13) are approximately 1 inch in size with grayish forewings marked with black spots and small black brick-like markings near the wing tips. The hindwings are marked with distinct patches of red, white and black. Adults lay yellowish-brown egg masses that are covered with a gray, waxy coating (Figure 14). The immature stages of the insect, or nymphs, are black with white spots, and they eventually develop bright red patches as they near adulthood (Figure 15).

Damage

Spotted lanternfly adults and nymphs often aggregate in large numbers on host plants.

Nymphs are known to feed on a wide range of plant species; however, adults prefer feeding and laying eggs on tree-of-heaven (*Ailanthus altissima*), particularly in the late summer and fall. The spotted lanternfly injures host plants by sucking the fluids from plant tissue, threatening

a plant's health. If left unmanaged, it can eventually kill the plant. They also produce copious amounts of a sugary liquid called honeydew, which can promote the growth of a black-colored fungus called sooty mold, and attract other insects.



Figure 14. Spotted lanternfly egg masses. (Photo credit: Pennsylvania Department of Agriculture, Bugwood.org)

Control

Currently, quarantines have been established in counties with infestations to stop or slow the spread of spotted lanternfly into new areas. In addition, efforts are being taken to ensure that this insect is not present outside quarantine zones. However, if you find an insect that you believe is the spotted lanternfly, collect a specimen or take a high-quality photograph, and contact the West Virginia Department of Agriculture or local WVU Extension Service office.



Figure 15. Spotted lanternfly nymphs. (Photo credit: Lawrence Barringer, Pennsylvania Department of Agriculture, Bugwood.org)

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