Seeking Biocontrols to Enable a Long-term Solution for Sahara Mustard (*Brassica tournefortii*) Invasion in North American Deserts

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J. David Garmon, MD,[1] Carl Bell,[2]
Travis E. Huxman, Ph.D.,[3] Jon P. Rebman, Ph.D.,[4]
Robert L. Staehle,[5] Lori L. Paul, RVT[6]

The estimated damage from invasive species worldwide totals more than $1.4 trillion – 5% of the global economy.
— The Nature Conservancy

Sahara mustard (*Brassica tournefortii*) is rapidly decimating North American deserts in the United States and Mexico by entirely replacing native wildflowers and vegetation, including creosote and cactus, with dense fields of mustard. This invader is highly adaptable and resilient, allowing it to swiftly expand its range through aggressive survival strategies. Sahara mustard first arrived in the United States in the 1920's (documented by J. B. Feudge, #1660, RSA, Feb. 1927) presumably as a contaminant with date palms imported from the Middle East into California's Coachella Valley.

Since 1997, Sahara mustard's expansion has dramatically accelerated, gaining a chokehold on once-vibrant wildflower fields in the Anza Borrego Desert and other regions of the greater Mojave and Sonoran Deserts. While hand-pulling and close surveillance has gained the upper hand against this invasion in limited areas such as Tubb Canyon (near the town of Borrego Springs, California), an effective biocontrol method is required to produce a long-term solution. We propose to take the first definitive steps toward effective biocontrol of this pernicious species.

Sahara mustard exhibits phased germination (not all seeds present in a single location will germinate at the same time), allowing it to get a head start on shading out native seedlings and to take advantage of intermittent optimal weather for seedling growth. Sahara mustard "steals" subsurface water from adjacent plants through a very deep, carrot-like taproot while its leaves are shaped to direct rainfall towards its central core and away from plants under its leaves or around

[1] President and Founding Director, Tubb Canyon Desert Conservancy
[2] University of California, Cooperative Extension, Regional Advisor—Invasive Plants
[3] Director, Center for Environmental Biology, University of California, Irvine
[4] Curator for Botany, San Diego Natural History Museum
[5] Founding Director, Tubb Canyon Desert Conservancy
[6] Founding Director, Tubb Canyon Desert Conservancy

its perimeter.

Individual Sahara mustard plants quickly produce flowers and myriad seedpods. Even when uprooted and left on the ground the fleshy taproot can continue to nourish the plant and complete its life cycle, producing thousands of seeds in just a few weeks. A large mustard plant can produce over 16,000 seeds, each with a sticky, mucilaginous surface. Seeds are dispersed via wind (often after the parent plant dies and becomes a far-ranging tumbleweed) and by adherence to wildlife (including bird feet and feathers), human footwear, and vehicle tires; thus enabling unintended transport of Sahara mustard seeds to new locations.

Few animals eat Sahara mustard, and none in large quantities due to its high oxalic acid content and the tiny, stiff urticating hairs on stems and broad leaves. Conversely, many desert natives are browsed by both vertebrates and insects, including voracious caterpillars during periodic mass irruption cycles. As a consequence, type conversion of native flora to a monoculture of Sahara mustard results in a desert "silent spring" where the diversity of wildlife is drastically reduced -- from insects, amphibians and reptiles to small and large land mammals, songbirds (including quails), and even raptors -- with many native species completely extirpated due to loss of either forage or forage-dependent prey.

Loss of spectacular desert wildflowers, iconic desert wildlife, and scenic vistas to Sahara mustard threatens the economic survival of communities that derive significant income from tourism.

Unfortunately, because this invasive species had been present at low densities in desert washes for decades (since at least 1927) without significant ecological damage, and because Sahara mustard was not a direct threat to agricultural crops, no immediate action was taken when a devastating change seems to have occurred around 1997, reportedly in Riverside County, California. Suddenly, Sahara mustard began exploding beyond isolated washes and rapidly began moving deep into native desert vegetation, often radiating out from roadsides and popular trails. It also began appearing at higher locations on hillsides and among boulder fields where the plant had not been previously observed.

Park agencies and other desert landowners sounded an alarm about Sahara mustard's dramatic expansion around 2003, but lacked adequate funds and dedicated staff for a swift response to this impending natural disaster. Insufficient research and belated direct action did little to slow, much less halt, the ever-expanding encroachment of Sahara mustard into fragile desert ecosystems. This lack of response grew critical by the mid-2000s when Sahara mustard began taking over pristine wildflower fields and popular recreational destinations. See the 2008 (before) and 2010 (after) photos, taken in Anza-Borrego Desert State Park.

BEFORE ➤

The 2008 wildflower bloom along Henderson Canyon Road in the Anza-Borrego Desert *before* take-over by Sahara mustard.

AFTER ➤

Same location two years later after Sahara mustard infested the wildflower fields in 2010. Note the dying creosote bushes.

(Before and After photos courtesy of Ron Neibrugge)

Control of Sahara mustard with herbicides is possible in limited situations, but not over the vast expanses this weed now inhabits in fragile desert ecosystems, ranging from the Torrey Pines of coastal California to the deserts of west Texas, from Utah to central Mexico. In a desperate holding action initiated by Tubb Canyon Desert Conservancy (TCDC) in 2011 in collaboration with AmeriCorps, Anza Borrego Desert State Park, the Anza Borrego Foundation, and other partners, Sahara mustard has been largely eliminated from a few accessible sectors of desert by intensive hand-removal of maturing mustard plants, followed by careful surveillance. However successful on a small scale, this labor-intensive approach will not in the long run win the war against this foreign invader.

Sahara Mustard has become an ecological disaster across the entire desert Southwest—from the coast of California to the deserts of west Texas, from Utah into central Mexico. What options remain?

Biocontrol

Biological control programs have proven to be an effective method that can reduce or eliminate populations of ecologically and agriculturally harmful invasive species. While manual methods of removing Sahara mustard have proven effective in relatively small, well-defined areas, human-power and herbicides are insufficient to halt, or even slow, the extent, or the current rate, of destruction of native desert ecosystems by Sahara mustard. *The only realistic hope of reversing the progress of Sahara mustard in the United States at this point in the invasion lies in discovering the biological agents that keep this species in check in its native habitat*. Sahara mustard remains in balance within its native range that extends from southern Europe and North Africa into the near East.

In spite of notable failures involving poorly planned release of biological control agents (the introduction of giant cane toads in Australia and mongooses to Hawai'i come to mind), biological control of highly destructive invasive species, when properly researched and carefully applied, have been very successful.

In the case of invasive plants, the track record is very good of getting control of the weed without coincident damage to native and useful (i.e. crops, landscape ornamentals) plants. For example, alligator weed (*Alternanthera philoxeroides*) from South America was introduced into North American bogs and waterways where it quickly formed impenetrable, spreading mats of growth in streams and lakes that prevented light penetration and reduced oxygenation, killing native aquatic plants, fishes and other aquatic species. Alligator weed also provided ideal habitat for breeding mosquitoes and clogged irrigation and flood control facilities. In an early, 1963 biocontrol program, the alligator weed flea beetle (*Agasicles hygrophila*) was released in Florida, and brought this invasive weed's population under control. Because of this success, and subsequent organisms released that feed on alligator weed, Florida was able to ban the use of highly toxic herbicides that had formerly been used to control alligator weed only three years after the flea beetles had been introduced. [http://www.sms.si.edu/IRLSpec/Agasicles_hygrophila.htm]

Highly invasive species like Sahara mustard (*Brassica tournefortii*) for which herbicides are not a viable option and which are "immune" to native control factors in an introduced environment, are extremely challenging to control and unlikely to be totally eradicated by stop-gap measures such as hand-pulling. Furthermore, the longer the control of this foreign plant is delayed, the greater the loss of habitat and the larger the possibility of reaching a "tipping point" where ecological catastrophe results and native biodiversity is overwhelmed. Sahara mustard's rapid takeover of native desert plant regimes, including annual wildflowers fields, in both low and high deserts, drives an urgent need to identify effective biocontrol agent(s). In order to find useful biocontrol agent(s) in the shortest possible time, detailed DNA analyses of Sahara mustard populations in both the United States and in the species' native range (Saharan Africa and the Middle East) are required as soon as possible.

Carpe DNA

Advances in DNA sequencing over the last two decades have fundamentally changed the process of locating biocontrol agents for invasive species. Current technology provides an unprecedented opportunity to pinpoint exactly where in its vast native habitat the Sahara mustard now spreading across North American deserts originated, thereby enhancing our ability to thoroughly search in that region for effective biological agents that will reduce the threat of Sahara mustard to our desert ecosystems.

Prior to the application of DNA sequencing, the process of looking for biocontrol agents was little more than trial and error, often under haphazard conditions in the field. Researchers would search the native range of an invasive species, which could cover tens of thousands of square miles and contain many populations of a particular species, in hopes of locating a biological agent that appeared to be keeping some populations of the invasive species in check. It would then take years to test the newly found biological agent(s) against the invasive species found in the non-native location. If a found agent was not effective against the particular population of the invasive species, it was back into the field to repeat the entire expensive and lengthy process over, and possibly over, again.

The obvious downside to this hit-and-miss approach is that, unless one is very lucky, it can take many years of expensive searching and testing to find a biological agent that is effective against a specific population of an invasive plant, particularly if that invasive species has a native range covering millions of square miles. If one is not lucky (and how often does the marble land in Red 36 on the roulette table?), this process could take decades to find an effective biologic agent. In the case of Sahara mustard, that is likely to be decades too late.

DNA sequencing has brought a laser-like focus to what was until very recently a trial and error process. Using DNA sequencing, it is now possible to pinpoint the specific, genetically identifiable population that the invasive species came from, and therefore its specific geographic origin, which provides a targeted location for biocontrol searches.

The modern process begins with a Phase I genetic analysis of hundreds of samples of the invasive species from the non-native habitat, i.e., the place where it is causing habitat destruction, such as the Southwestern United States. This genetic analysis produces a number of critically important pieces of information, the first being to establish the level of homogeneity in the invasive population. Homogeneity refers to the genetic similarity of all the plants being tested. If the tested plants are identical in their genetic sequence, they are said to be homogenous and therefore are all descended from a common origin.

Another possible finding from the DNA sequencing of Phase I is that the invasive plants may represent two or more populations of the same plant species but with different geographic origins. Each population of plants would be homogenous within itself but genetically distinct from its close relatives. The implication here is that the invasive species was introduced to the non-native range on more than one occasion and from more than one point of origin.

Phase II of this process begins in much the same way as Phase I, except that this time the DNA being sequenced is from plants in the native range, such as Europe or North Africa. If the native

range is vast, as is the case with Sahara mustard, we would expect to find subtle, yet distinct, genetic differences among the populations of Sahara mustard throughout its native range. This is to say that Sahara mustard found in Afghanistan, for example, would be the same species of plant as the Sahara mustard found in Tunisia; but we would be able to now genetically distinguish these two populations of the single Sahara mustard species. Depending upon the size of the native range and the number of plants sequenced, we could expect to see dozens of genetically distinct populations of plants that we would be able to map to specific geographic locations.

This leads us now to the power and specificity of modern DNA sequencing. *Armed with the results of the genetic sequencing of the invasive plant conducted in Phase I, we can now compare that genetic sequence to the specific DNA sequences of the populations in their natural habitat from Phase II. When we match the genetic sequence from the invasive plants with the genetic sequence from one or more of the native plant populations we will then be able to pinpoint the geographic origin of "our" type of Sahara mustard. Given that information, we will then know exactly where to look for biocontrol agents.* No more guessing, no more trial and error, no more botanical roulette.

Cost-effective DNA Sequencing

Twenty years ago the cost of a comprehensive botanical DNA investigation would have been prohibitive. However, the process of DNA sequencing that was once on the cutting edge of science has become automated, routine, and inexpensive. *The sequencing of one plant sample that once would have cost thousands of dollars and hours of a researcher's time can now be done for less than $10.*

Testing

Once biological agents that control Sahara mustard in its native habitat are identified, there is an elaborate protocol that has been used for decades to test an agent's effectiveness in the laboratory and in the field. An equally important aspect of this testing is to assure that the discovered biocontrol agents do not "misbehave" if released in a new environment such as the deserts of the Southwestern United States and arid northern Mexico. This latter aspect of testing will be particularly important in the case of Sahara mustard because of its close genetic relationship to a number of commercial crops, notably kales, cabbages, Brussels sprouts, broccoli, and cauliflowers.

Action

We propose work in three phases over three years. This work will represent the first steps toward finding and implementing an effective biocontrol method to stop the aggressive spread of Sahara mustard and its consequent destruction of vulnerable, beautiful, and economically important native desert habitat.

Phase I (2014 June through 2015 May)

1. Perform DNA sequencing ("destructive sampling") of Sahara mustard already collected, and housed in herbaria, from the current geographic range of Sahara mustard across the desert Southwest of the United States.
2. Acquire and analyze new samples of recent mustard growth in the Anza Borrego Desert and surrounding areas, which have been the subject of recent study and selective hand removal.
3. Determine the genetic homogeneity / diversity of the invasive population(s), thereby revealing whether Sahara mustard in North America originates from one source location in the plant's native range, or several. We will also be able to determine what degree of hybridization among populations has occurred among this invasive mustard?

Work to be performed by: University of California, Irvine and affiliated laboratories under the direction of Travis E. Huxman, Ph.D., Director, Center for Environmental Biology, UC Irvine and Director or the Steele/Burnand Anza-Borrego Desert Research Center.

Results to be published in scientific literature and online in a format useful to land stewardship agencies (parkland managers), universities, botanical organizations and research institutions, including State/Federal academic agricultural extensions.

Estimated cost: $60,000

Phase II (2014 September through 2015 July)

1. Ascertain the locations and contacts for overseas herbaria with collections of Sahara mustard from its native range upon which DNA sequencing can be performed.
2. To the extent needed to fill probable gaps in existing overseas collections, acquire new samples (direct field collection) from native Sahara mustard populations in as many locations as possible.
3. Perform genetic analysis in alignment with analysis performed in Phase I, Steps 1 & 2.
4. Correlate results in Phase I, Step 3 with Phase II, Step 3 to determine geographic range and site(s) of origin for the invasive mustard populations found in the Southwest United States and northern Mexico.

Work to be performed by: USDA-ARS- European Biological Control Laboratory, Montpellier, France under the direction of Marie-Claude Bon, Ph.D.

Results to be published in scientific literature and online in a format useful to land stewardship agencies (parkland managers), universities, botanical organizations and research institutions, including State/ Federal academic agricultural extensions.

Estimated cost: $60,000

Phase III (2015 August – ~2018)

1. Create a biocontrol testing, introduction, and implementation plan based on results of Phases I and II.
2. Laboratory and sequestered field testing per an approved plan.
3. Introduction based on an approved plan and changes in the plan based on the results of testing and regulatory approvals.
4. Implementation based on above that will reduce Sahara mustard under field conditions.

Work to be performed in association with: USDA-ARS Exotic and Invasive Weeds Research, Albany, CA, and/or CA Department of Food and Agriculture Biological Control Program.

Land stewardship agencies: US Fish and Wildlife Service, US Bureau of Land Management, US Bureau of Reclamation, CA Department of Parks and Recreation, CA Department of Fish and Wildlife.

Appropriate regulatory bodies: CA Department of Food and Agriculture, US Fish and Wildlife Service, CA Department of Fish and Wildlife.

Costs and funding sources for Phase III will be estimated based on results of the two prior Phases. Costs and parties affected by *not* taking action will be estimated based on observed progress of the Sahara mustard invasion and type conversion, associated environmental damages and adverse impacts on affected communities (including recreational parklands), and comparison with similar invasive species impacts in other ecosystems.

For further information, contact:

David Garmon, President
Tubb Canyon Desert Conservancy
www.TubbCanyonDesertConservancy.org
800.428.8903 / +1 858.535.9121

8899 University
San Diego,
USA

jdgarmon@mac.com
Center Lane, #170
California 92122