

Plants' Suitability for Bt-Crops with Insect Resistance

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Abstract

When field trials of transgenic plants are hampered by practical containment problems, manipulation experiments can uncover the potential consequences of cultivated and wild gene flow. Using Bt *Bacillus thuringiensis* topical larval spray and larval supplements, we measured the effect on herbivore fitness reduction on *Brassica rapa* and wild radish *Raphanus sativus*. These species represent different life histories among potential Bt transgenic recipients from Bt cabbage in the United States and Asia, where rare random mating is expected with high exposure. Protected wild radish and wild mustard seedlings had about half of the herbivore damage compared to exposed plants and a 55% lower seedling mortality rate, resulting in successful reproduction. 27% higher yield, 14 days longer shelf life and 118% more seeds on average. Seed addition experiments in miniature and in situ indicate that wild radish is more contagious than wild radish in coastal grasslands.

Keywords: Seed; Grassland; Vegetation; Incompatible

Introduction

Commercial GMO and insect-resistant crops currently grown in the United States have virtually no wild relatives near the production site, ensuring that new crop traits are unlikely to migrate through the wild gene pool. local barbarians. However, an assessment of the consequences of gene flow will be necessary in future deregulation decisions as most of the world's major and minor crops either exist in the wild or crossbreed with them wild goods somewhere in their range [1]. Wild relatives of transgenic plants acquire IR traits through gene flow, and penetrance can be released from the pressure exerted by sensitive herbivores. However, the lack of knowledge about the ecological factors regulating weed abundance, competitiveness, or geographic distribution has limited our ability to predict whether new plant defenses could potentially increase the weediness of the plant's wild relatives or even whether herbivores would have a negative effect positive effect on plant growth and condition. Surprisingly few tests have been conducted on the effects of herbivores on the spread of invasive plants or to quantify the effects of herbivores on plant survival. . The identification and quantification of environmental risks associated with gene flow from GM crops is subject to methodological compromises due to confinement limitations, especially with respect to the adaptive effects of the plant organisms, which require pollen production. Field trials with GM pollen-producing plants should be physically confined in cages or greenhouses or set up in locations where wild relatives are not found. Conditions in areas where there are no natural populations of wild relatives may differ from areas of concern for hybrid formation in ways that affect the results and therefore the extent to which hybridization is concerned. suitability of these field trials [2-5]. An alternative method, used in this study, is to perform in situ trials in which hybrids are expected to occur naturally, using complementary/protective techniques herbivores on non-transgenic wild plants. This method can be adapted to examine the adaptive effects of IR transgenes in cultivated wild hybrids worldwide, for example, the Bt-Brassica IPM programs in Asia and Asia Africa and the adoption of Bt maize in Mexico.

Materials and Methods

Study

We compared individual fitness parameters of wild Brassicaceae radish: *Raphanus sativus* L. 2003-2004 and wild radish and Brassicaceae:

Brassica rapa L. 2004-2005. In the central coast of California, both species are naturalized annually in winter, appearing as seedlings in October or November with the first rains. Wild radish flowers earlier and sets seeds in spring than radishes that flower and seed until August or September. Sometimes wild turnip plants survive for two years. Both wild mustard and wild radish are incompatible, pollinated by insects and belonging to the plant-weed-wild complex. *R. sativus* and *B. rapa* have a fixed seed bank and are common in agricultural fields, field edges and coastal grasslands [6-10]. These habitats vary in resource and quality of vegetation. Agricultural habitats are typically fertilized and irrigated, while adjacent grasslands receive water only from seasonal rainfall. The highest levels of disturbance are in arable fields, which are plowed to remove weeds and tilled before annual crops are planted. Wild radish and wild mustard are treated with herbicides or controlled by machines or manually weeded by local growers and managers of nature reserves, railway corridors, public parks and cities and wetlands.

Wild radish and mustard are hosts to a variety of herbivores, including cabbage aphids, green peach aphids, the flea *Phyllotreta cruciferae* L., the diamondback caterpillar *Plutella xylostella* L., the cabbage caterpillar *Pieris rapae* L., the cabbage beetle *Trichoplusia ni* L., slugs and snails. We have applied weekly bactericidal sprays to suppress tissue shedding of sensitive Lepidoptera such as *P. rapae* and thus create an herbicidal treatment for several insect species. eat these plants. All plants were sprayed weekly with 1g of Dipel Dry Flowable powder suspension per 750mL of plants protected with deionized water or a suspension of the same concentration from cooled Bt exposed plants as a added water control agent and microbial raw materials [11]. This Bt is a suitable mimic for Cry1, which is related to the herbivore of Brassica spp. and their relatives. Routine treatments are needed to simulate continuous dosage levels of Bt transgene expression or simply act as a herbivore exclusion treatment, because of the microbial insecticide

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class. the object is decomposed by UV rays and washed away by rain; This method may underestimate the insecticidal effect of transgenes in geographical areas with high rainfall.

Experimental Design

Hierarchical treatment design for the first year, with herbivorous treatments nested into habitat types at three levels, except in two places where seedlings are sparse in disc plots, nested in place. Ten wild radish plants were selected from each plot, totaling 2,600 plants and marked initially with numbered stakes and then with a numbered spiral linkage around the stem. Graft lots were randomly assigned to receive either weekly Bt spray on protected plants or denatured Bt spray and larval supplementation on exposed plants. An early stage of *P. rapae* was placed on each wild radish experimental plant at three equally spaced intervals from December to February, corresponding to the seedling to reproductive stages [12-14]. The percentage of herbivore damage per plot was estimated once, 12 weeks after the first rain, by specifying the damage type for each leaf of each plant and converting the average of categories per tree to the average percentage of this category. The total number of live radish seeds per small plant was counted after lightly pounding the pods. For large plants, the number of live radish seeds per 15 fruits was used to estimate the total number of seeds by weight.

Results

The background vegetation is already fully formed by the time of wild mustard and radish breeding, due to the germination and growth of annual plants in winter after the onset of autumn rains. At 12–13 weeks after the first rain, the average ground cover in all subplots was more than 90%, including the disc-shaped subplots, which initially had no ground cover. The average overall plant species richness was 6.8 ± 0.1 SE in all subplots, with no significant differences between habitat types. The mean bare land cover in disc plots did not differ significantly from field edge habitats but was still significantly higher than in natural vegetation habitats ($10.5\% \pm 2.2\%$ SE in natural vegetation compared to $9.4\% \pm 1.8\%$ SE at field edge vs. $6\% \pm 1.7\%$ SE in disc parcels) (GLM ANOVA per percent of bare ground cover $F = 3.15, P = 0.0450$). Experimental supplementation of wild radish and wild mustard seeds resulted in higher recruitment, even when the number of seeds per miniature or plot was relatively low, estimated at half of the additional seeds generated. produced by a medium plant in low herbivore treatment. The mean densities of wild radish were significantly higher when seeds were added to the soil compared with the no-seeded control, either in the original bare soil microarrays or in natural plant microbiomes with intact ground cover. Whole Species richness of other plant species emerged from the seed bank in the average $4.5 (\pm 0.3)$ SE herbaceous and grassy soil microbiomes, and natural plant microbiomes averaged $5.2 (\pm 0.4)$ SE other plant species, suggesting that seed addition makes a difference in wild radish recruitment for plant community development in different environmental types. this different habitat. Seed addition also significantly increased the average recruitment of wild mustard seedlings, but only in disc soil habitats. Unlike wild radish, the density of wild mustard is significantly affected by the type of dispersal habitat type compared to the wild type, so very few plants survive to flower in the microcosm of the carpet. natural plants. The species richness of other plants in these subuniverses was $4.6 (\pm 0.3)$ SE and $5.3 (\pm 0.3)$ SE other herbs and grasses, respectively. in plates and natural plant microspheres.

Discussion

Although there is general agreement that herbivores can have

pronounced negative effects on plant fitness, plants also exhibit resistance to herbivores and tolerance to herbivore damage. Thus, simple field tests have a place in directing further study and consideration of mitigation techniques toward the cases of expected transgene introgression which are most likely to increase the fitness and spread of weedy wild relatives in natural plant communities and agroecosystems. In our study, slight-to-moderate protection from herbivory, measured as the average difference between treatments on juvenile plants 5% on wild radish in year one, 17% on wild mustard in year two, and 16% on wild radish in year two, significantly increased lifetime seed output of an average plant to about double that measured for plants with greater mean herbivory. Typical fluctuations in herbivory, then, that routinely reduce plant fitness in the field under present conditions, may be nullified to a significant degree with the introgression of a Bt trait for antiherbivore defense. The result could cause average seed output to shift to a higher level, depending on population levels of susceptible herbivores. A significant proportion of plants protected in our treatments had some leaf tissue removed, for example, 45% of wild mustard plants sprayed with Bt in 2003-2004, showing that Bt-sensitive and non-sensitive invertebrates have consumed plant tissue. Lepidopteran-resistant Bt canola and possibly Bt broccoli or cabbage have the potential to cross with *B. rapa* and other wild relatives if grown commercially on the California coast. To examine the potential impact of body condition in two contrasting wild relatives in this study, we selected the two most abundant random parent species near cabbage fields, *B. rapa* and *R. sativus*, differing in most life-history features. Although little is known about *R. sativus*, tests with *R. raphanistrum* have shown it to be unlikely to cross with Brassica but see Gueritaine. Because gene flow between plants and weeds is highly specific and difficult to completely eliminate in many parts of the world and for many crops, research on the effects of transgenes on natural communities and References found there and field trials where the pest's wild relatives - Naturally occurring disease resistant plants are needed to assess the risk of transgenic crops. Whether stably expressed transgenes in cultivated and wild hybrids allow them to compete favorably, survive and propagate more rapidly depends greatly on the fitness advantage of the trait. resistance is introduced. Although wild mustard enhances its overall health when protected from a subset of Bt-sensitive herbivores, *B. rapa* protected by Bt is the least competitive cruciferous family in the regions. non-agricultural areas with established vegetation. The overall fitness advantage of *B. rapa* plants protected by Bt endotoxins and lower reactivity in established vegetation is similar to and complements Stewart's results when comparing Bt-Brassica napus transgenic and non-transgenic *B. napus* has undergone a deciduous phase resulting in a 40-60% increase in the percentage of herbivores, due to the addition of diamondback moth larvae to natural and cultivated plant patches. Similar increases in life cycle fitness of wild radish *R. sativus* with Bt spray compared with larval supplementation were stronger for habitat type differences compared with *B. rapa*, for found that fitness impact testing for wild relatives should involve representatives of a variety of species under different conditions. site conditions.

Conclusion

Tiered risk assessment frameworks are designed to make an informed prediction of the likelihood of harm resulting from an action, using a minimal amount of data. Using the framework proposed by Raybould and Cooper, the Bt-based resistance-related fit advantage test in wild plants constitutes a secondary risk assessment. One of the advantages of herbivore exclusion tests at this stage of the risk assessment process, which is designed to determine the potential for increased weediness in hybrid cultivars grown with invasive plant protection transgenes,

are tests that can be performed in areas and habitats where hybrids are likely to form. Another thing is that genetically engineered constructs from different wild relatives are not required. However, as with our wild mustard study, factors such as fertility and vigor of the hybrid, the occurrence of hybrid seeds, the difference between transgene expression and the spray at where, from the genetic load to the range of herbivore toxicity and competitiveness of F1 and backcross generations can partially mitigate or exacerbate any undesirable outcomes. resistance traits of the host plant. However, it is important to assess the weed-promoting capacity of plant species that exhibit new traits in different actual habitats, with a variety of potential environmental and economic consequences. Herbivorous exclusion experiments are therefore useful tools for predicting the potential impact of a wide range of insect resistance traits on the population dynamics of likely plant parents. traits through gene flow from transgenic plants.

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