Prerelease efficacy assessment, in quarantine, of a tephritid gall fly being considered as a biological control agent for Cape-ivy (Delairea odorata)

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Abstract

Due to the long-standing emphasis on only releasing host-specific agents, classical biological control of weeds has an enviable track record of few direct impacts to nontargets. However, even an agent whose host-range is restricted solely to the target weed can have indirect impacts. Such indirect impacts are most likely if, after release, the populations of the agent build up to high numbers without causing accompanying declines in the populations of the target weed. Therefore, it is advisable, prior to release, to demonstrate that the candidate agent is not only host-specific, but that it has clear potential to depress populations of the target weed. Prerelease efficacy assessments (PREA) of potential weed biocontrol agents are not yet common, and are most easily done in the region where both the target and the potential agent are native. We present an example of a PREA performed under strict containment conditions of an approved quarantine facility. A gall-forming fly, Parafreutreta regalis, from South Africa is being considered for release in California to control Cape-ivy, Delairea odorata. We conducted two trials exposing test Cape-ivy plants to two different densities of this fly, and, after approximately two months, comparing the growth of the galled vines to similar vines that had not been exposed to flies. Under both the high density (10 pairs of flies/plant) and low density (2 pairs/plant) treatments, the galled vines exhibited visible stunting, and the ungalled stems were longer, and had more nodes and larger leaves. These trials confirmed that relatively subtle, sublethal impacts on the target can be quantified, even under strict containment conditions, and this should encourage others to assess, prior to release, the potential impact of prospective agents on their proposed target.

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1. Introduction

Classical biological control is considered by most practitioners to be an environmentally benign approach for controlling invasive, nonindigenous pests. Classical biological control of weeds has an exceptional track record for successfully controlling invasive plants and minimizing negative impacts to nontargets. Although more than 350 organisms have been released to control 130 weed species worldwide, only eight examples of impacts on nontarget plants are known, and most of these were predictable from the prerelease tests (Julien and Griffiths, 1998; McFadyen, 2000; Pemberton, 2000; Louda et al., 2003a,b; Willis et al., 2003). This enviable record of safety is due primarily to the long-standing concern by weed biocontrol scientists about the host-specificity of the agents that they release (McFadyen, 1998). All of the five nations that are responsible for the majority of weed biocontrol releases globally use the results of host-specificity tests as the cornerstone of their respective permitting processes (Sheppard et al., 2003).

While host-specificity tests have proven their value in predicting and minimizing direct impact on nontarget plants, they are of little value in predicting possible indirect effects. Intentionally adding an exotic organism into an ecosystem might result in ecosystem perturbations, even though the organism displays great fidelity to its host, and it never attacks or directly harms any nontarget organisms. For example, the introduced agent might provide a new
food resource for predators or parasites. This concern about possible indirect effects has been largely theoretical (Simberloff, 1991; Simberloff and Stiling, 1996), but recent studies in Montana of two flies, Urophora affinis Frauenfeld and U. quadrifasciata (Meigen) (Diptera:Tephritidae), that were released to control spotted knapweed (Centaurea stoebe micranthos (Gugler) Hay) (Asteraceae), (often referred to as Centaurea maculosa Lamark) indicate that these flies may be having widespread and significant impacts on mammals and other organisms (Pearson et al., 2000; Pearson and Callaway, 2005, 2006). These flies were released in North America in the early 1970s, have become widespread on their host, and in Montana one or both can be found at almost all knapweed sites (Story et al., 1987). The larvae of these flies overwinter in galls that they induce within the flower heads of spotted knapweed, and although they cause seed reduction of 36–41% in the heads of spotted knapweed in Montana (Story et al., 1987), they have failed to control this weed (Myers and Harris, 1980; Maddox, 1982; Julien and Griffiths, 1998). Deer mice, Peromyscus maniculatus (Wagner), have learned to exploit the abundant overwintering larvae, which have now become their primary winter food source, and they can consume several hundred larvae per day when they are available (Pearson et al., 2000). This has resulted in high populations of deer mice in knapweed-infested areas, at the expense of other rodents such as voles (Pearson, 1999; Ortega et al., 2004). Since deer mice harbor hantavirus (Childs et al., 1994; Nerurkar et al., 1994), there is speculation that increased populations of these rodents and the higher levels of hantavirus among these mice may lead to a greater incidence of this serious pulmonary disease in humans (Pearson and Callaway, 2006). However, if some combination of the 13 different biological control agents that have been released against spotted knapweed (Julien and Griffiths, 1998) are successful in reducing this weed’s populations, then the Urophora populations would also decline, and no longer provide a significant food subsidy for field mice. There is evidence that some populations of spotted knapweed in Montana are now starting to decline due to impacts of some of these other, more recently released agents (Story et al., 2006). There is also evidence that these more recently released knapweed biocontrol agents are reducing populations of the Urophora flies in Colorado (T. Seastedt, Univ. of Colorado., Boulder, CO, personal communication). Even though Urophora fly populations might be declining, the more dire predictions by Pearson and Callaway (2006) are gaining public attention. A recent article in the New York Times quotes a noted ecologist as saying, without supplying additional examples, “What Dean (Pearson) has found is the tip of the iceberg.” (Robbins, 2006). Clearly, weed biocontrol practitioners need to be concerned about not only direct impacts of their agents, but also potential indirect impacts. Indirect impacts, such as those that Urophora flies are having on deer mice, are difficult to predict prior to release. However, with additional testing, beyond the traditional host-specificity tests, the probability of such indirect impacts can be reduced. Indirect impacts from a biological control agent are most likely to occur if the agent becomes very abundant (Holt and Hochberg, 2001; Balciunas, 2004a; Pearson and Callaway, 2005). While biocontrol specialists hope that, after release, the agent will establish and become abundant, high populations of the agent should be followed by the collapse of the populations of the target pest, and subsequent decline of the agent. A candidate agent that has demonstrated its ability to kill the target weed might not need further documentation of its potential impact. However, a candidate agent whose attack on the target weed causes sublethal effects, should be evaluated, prior to release, to demonstrate that it has potential to reduce populations of the target weed (Sheppard, 2003; Balciunas, 2004a; McClay and Balciunas, 2005; Pearson and Callaway, 2005). Recent theoretical studies indicate that prerelease efficacy assessments (PREA) to determine a candidate agent’s potential impact are cost effective, and should be conducted before host-specificity studies begin (McClay and Balciunas, 2005). The need for releasing effective agents has been acknowledged by the practitioners of weed biocontrol. Guideline 3 of the “International Code of Best Practices for Classical Biological Control of Weeds” calls for practitioners to “Select agents with potential to control target” (Balciunas, 2000; Balciunas and Coombs, 2004).

While, in most of the developed countries that are involved in the introduction of new weed biocontrol agents, host-range testing is now required (Sheppard et al., 2003), prerelease efficacy assessments are not. PREA are probably most easily performed under noncontainment conditions, in the region where the weed and the candidate biocontrol agent are both native. For example, in Australia, the home of melaleuca (Melaleuca quinquenervia (Cav.) Blake), Balciunas and Burrows (1993) used insecticides to exclude insects from attacking the ‘control’ saplings of melaleuca, and demonstrated that sprayed saplings quickly outgrew those that were unprotected, and were able to infer that two insect species were likely responsible for this suppression of sapling growth. Studies in Europe, prior to their release in Australia, confirmed the potential negative impact of root-feeding insects on Carduus nutans L. (Sheppard et al., 1995), and of the weevil Lixus cardui Olivier (Coleoptera:Curculionidae) on Onopordum spp. thistles (Briese, 1996). In another, more recent, study in Australia, Goolsby et al. (2004) showed that an Australian mite, Floracarus perepae Knihinicki & Bozek (Acariformes:Eriophydae), being considered as a potential biocontrol agent for Lygodium microphyllum (Cav.) R. Br. in the USA, dramatically reduced the biomass of this invasive vine.

However, conducting PREA’s in the native range can have drawbacks. Other herbivores and pathogens cannot always be excluded, and the candidate agent’s populations may be limited by parasites or predators. Frequently, it is not feasible to conduct a PREA in the native range of the target pest. Doing so under the cramped conditions, with limited numbers of the agent, and under strict containment...
protocols of a quarantine facility poses additional challenges. The purpose of this study was to determine, in our USDA-ARS quarantine in Albany, California, if a gall-forming tephritid fly, *Parafreutreta regalis* Munro (Diptera:Tephritidae), causes sufficient damage to Cape-ivy, *Delairea odorata* Lemaire (Asteraceae), to warrant further consideration as a potential biological control agent for this pest. The number of flies and cages that could be used was very limited, so, by necessity, the experimental design needed to be small and simple. Likewise, we did not want to interrupt the ongoing host-specificity evaluations of this fly for longer than necessary, and decided to devote only half a year to conducting these PREA trials. If the PREA trials convinced us that this fly could impact the growth of Cape-ivy plants, we would complete the host-specificity testing. If not, we were prepared to terminate our colony of these flies, and devote our time to other potential agents.

Cape-ivy, also known as German ivy and *Senecio mikanoides* Otto, is an ornamental vine, native to South Africa (Hilliard, 1977). It has escaped from cultivation and has become a serious pest in coastal regions of California and upland Hawaii in the USA and several other countries, including Australia, England, Italy, and Spain (Bossard, 2000; Robison et al., 2000; Balciunas, 2004b). Cape-ivy is a winter-flowering perennial, whose shoots form mats on the ground and clamber up adjacent vegetation, frequently smothering it. In California, this vine is most invasive and widespread in riparian areas and coastal scrub communities.

A biological control project targeting Cape-ivy was launched in 1998, and surveys in South Africa indicated a half-dozen potential biological control agents (Grobbelaar et al., 2003). Prior to requesting release in California, we are evaluating the host-range of *Parafreutreta regalis* (Diptera:Tephritidae), one of the few insects described from Cape-ivy (Munro, 1940). A female *P. regalis* oviposits several to many eggs in a node or growing tip of Cape-ivy.

A month after oviposition, the gall is fully formed, and the larvae chew a small, circular “window,” covered by the intact plant cuticle, on the side of the gall (Fig. 1). After another month, adult flies, usually 5–12 in number and slightly smaller than a house fly, break the “window” and begin emerging from the gall. The life cycle from oviposition to adult emergence is approximately two months in our laboratory, but requires an extra week or two during winter in our laboratory. The female will begin to oviposit within a day or two of emergence, and the adults live for approximately two weeks. Under laboratory conditions, we did not observe any diapause. We chose to conduct each of our trials for two months, the duration from oviposition through the emergence of the first adult flies. This would allow the galls to develop on the vines, and we hoped that we could detect the impact that these galls had on the structure and growth of Cape-ivy vines.

2. Materials and methods

The assessment of the possible impact that *P. regalis* might have on Cape-ivy was conducted as two separate trials, each with a different density of flies. Both trials were performed under containment conditions in the USDA-ARS weed biocontrol quarantine facility located at Albany, California, USA. During the trials, although supplemental heating and cooling were used, ambient temperatures ranged widely, from 11 to 36°C. The trials were conducted during winter and spring, when Cape-ivy grows most vigorously, and natural lighting was supplemented by four 200-watt incandescent bulbs, about 1.5 m above the plants, that were turned on from 6 am until 8 pm daily. From March through mid-May, the lights were turned off from 10 am to 4 pm.

The flies used in the trials were from our laboratory culture that was started from galls originally collected at the town of Wilderness, in Eastern Cape Province, South Africa.
The Cape-ivy plants used in the trials were grown from cuttings in our greenhouse, from material originally collected at Rocky Creek, 20 km south of Carmel, California. In California, two varieties of Cape-ivy are found. One has ear-like, flattened stipules (sometimes referred to as auricles) at the base of leaf petioles, while the other variety lacks these stipules. Only the stipulate variety, the most common variety in both South Africa and California, was used in the impact trials (Balciunas, unpublished data).

For the first trial, we selected 10 small, single-stemmed Cape-ivy plants growing in pots with stakes, from our greenhouse cultures. They were similar in size (approx. 25-cm long) and number of leaves (10–15). On December 24, 2001, a pair of these plants was placed into each of two plexiglas sleeve-cages (56 cm wide, 40 cm deep, 21 cm high) equipped with two cloth sleeves. Into the first cage, 40 newly emerged *P. regalis* (20 females and 20 males) were released, while the second cage served as a control and contained no flies. Two days later, when more newly emerged flies were available, three more cages, each containing two Cape-ivy plants, were set up. One of these served as another control and had no flies, while 20 pairs of flies were released into each of the other two cages. The plants were watered twice per week. Every weekday morning, the five cages were inspected and the number of live flies observed. Any dead flies were replaced with fresh flies of the same sex. Sometimes the flies died, and their deaths went undetected. Whenever the count of flies observed in a cage dropped by 50% (10 flies), all flies in the cage were collected, and each plant carefully examined for additional dead or living flies. Then the live flies were released back into the cage, and additional flies added to bring the count back up to 20 male/female pairs. A nutrient source (50% Mountain Dew™, PepsiCo, Inc.) was provided for the flies, and the plants were watered as necessary, by inserting a beaker of water through the cage’s sleeve. Likewise, any aphids or other less common pests that were observed were crushed. On February 15, 2002, the first fly emerged from a gall on a test plant. All flies were then removed from the three test cages, but the test and control plants were kept in cages for an additional four weeks, to allow full gall development. During this period, any emerging flies were removed daily.

On March 12, 2002, all the plants were cut off at soil level, the length of their shoots measured, the number of nodes counted, and the width of the primary stem measured at 10, 20, and 30 cm above the soil. The galls from each plant were then removed, counted, measured, and weighed. The galls were then dissected and the larvae, pupae, and/or empty pupal cases counted. The leaves were removed from the stems, classified as either small (blade length less than 2 cm) or large (>2 cm), and the fresh leaves and stems were weighed. The roots of Cape-ivy vines were not weighed because they are fibrous, and difficult to completely separate from the potting medium.

The second trial was similar to the first, but used a lower density of flies (2 male/female pairs per plant) and larger plants with shoots averaging 33 cm in length. Prior to Fig. 2. The appearance of the 10 Cape-ivy plants at the conclusion of Trial 1. At the start of the Trial, plants were ‘paired’ for similar size and number of leaves. The six ‘treated’ plants (1T, 2T, ..., 6T) were each exposed to 10 pairs per plant of *P. regalis* gall-forming flies for 63 days in Trial 1. The four control plants (1C, 2C, 3/5C, and 4/6C) were kept in similar cages and not exposed to flies (see Section 2). One of the control plants (2C) developed a gall (see Section 3), and was excluded from the analyses. For each pair, note the shorter length and the fewer leaves on the galled plants, when compared to the ‘control’ Cape-ivy plant on the right.
initiating Trial 2, six pairs of single-stemmed plants were selected, their shoot lengths measured, and the number of nodes and small and large leaves counted. Since the stem diameters were unaffected by the galls in Trial 1, they were not measured in Trial 2. One of each pair of plants was designated a control, the other a test plant. Analysis (Student’s *t* test, (Statistix, 2005)) of the mean pooled shoot lengths, as well as the number of nodes, and small and large leaves, for the controls and test plants confirmed that controls were statistically indistinguishable from the test plants at the start of Trial 2. All 12 plants were provided with small trellises upon which the vines were trained as they grew. Initially, the six test plants were placed, two each, into three plexiglass sleeve-cages, and the six control plants in three other cages. On March 4, 2002, four male/female pairs of flies were released into each of the three cages containing test plants. The flies and plants were observed and treated as in Trial 1. However, after three weeks, it became apparent that some of the plants had already outgrown the sleeve-cages, and that their growth was being restricted. Therefore, on March 28, 2002, all the flies in the three test cages were captured, and the six test plants transferred into one large (92 × 92 × 122 cm), metal, screened cage, into which 12 pairs of flies were also released. The six control plants were likewise transferred into an identical cage without flies. On May 2, newly emerged flies were observed in the test cage, and all the flies were removed. A week later, the test plants and control plants were measured in the same manner as in Trial 1. The galls were inserted into a block of moist florist’s foam, and held for another month to allow the development to proceed, then dissected, and the number of immatures and empty pupal cases counted.

For both trials, the data were analyzed by analysis of variance (ANOVA) using Super ANOVA (Gagnon et al., 1989). All count data were first transformed by square root (Y), or by square root (Y + 0.5) for data with zero values, and proportion data by arcsine square root (Sokal and Rohlf, 1981). For both trials, the initial ANOVA model included treatment and cage-within-treatment effects.

### 3. Results and discussion

In the first trial, within a few weeks the exposed plants all had several nodes that were beginning to swell, indicating that galls were beginning to form. By the time the first trial was ended after two months, the six Cape-ivy plants that were exposed to flies were all noticeably smaller than the four plants kept free from flies (Fig. 2). The six test plants developed a total of 12 mature galls and 28 incipient galls. Mature galls are those that enlarged and developed to the point that they were capable of producing adult flies, while incipient galls showed signs of swelling, but failed to produce mature larvae, pupae, or adults. Mature galls were much larger, and accounted for 84% of the total gall biomass during Trial 1. A single moderately large gall might account for 15–20% of a small vine’s weight, and plant 3T had 41% of its aerial biomass devoted to galls. The two most stunted vines (5T and 6T) failed to develop mature galls. Dissections of these two plants indicated that all the nodes had multiple ovipositions. It is likely that the neonates and young larvae were killed by subsequent oviposition attempts at the same node, but this repeated oviposition and partial development of immatures was enough to severely inhibit growth of these two vines. One of the control plants (2C) also developed a gall. It had been removed from its cage briefly so that the cage could be repaired. During this interval, a female *P. regalis* that had escaped from an adjacent host-specificity test had oviposited on it; we excluded this control plant (2C) from our statistical analyses.

![Graph](image-url)

**Fig. 3.** Mean plant-measurements of 6 ‘treated’ Cape-ivy plants after 2 months of continuous exposure to 10 pairs per plant of *P. regalis* gall-forming flies, and 3 ‘control’ plants not unexposed to the flies in Trial 1 (±SE). *—means were significantly different from treatment (ANOVA, *P* < 0.05).
In Trial 1, the ANOVA indicated that the cage-within-treatment effect was not significant \( P > 0.05 \) for any of the response variables, so this term was removed from the ANOVA model for final analysis. Even though the sample sizes are small, the statistical analyses confirm the visible stunting of the galled plants. Of the 10 plant characteristics measured, four were significantly lower for the galled plants when compared to the three ungalled plants (length of primary stem—55% shorter; length of all stems—56% shorter; stem weight—36% lighter; number of nodes—52% fewer; number of large leaves—69% fewer; and total leaf weight—48% lighter) (Fig. 3). Total aboveground weights of the plants were not significantly different \( P = 0.399 \), indicating that galling did not reduce aboveground plant biomass, but that resources were allocated to forming galls, at the expense of stem elongation and developing mature leaves.

The number of small leaves (<2 cm) on the galled plants was almost three times greater than on the ungalled (25.8 vs. 9.7). Because of the large variation in the number of small leaves among the 6 test plants, this large difference was not significantly different in the ANOVA. However, when we ran a regression analysis between the number of galls and the number of new leaves, the number of new leaves increased as a function of the number of galls on a plant \( Y = 2.86 \pm 0.78 \) (SE) \( X + 7.7 \) (±4.8)) and this was highly significant \( F_{1,7} = 13.5, P = 0.008 \). We believe that this abundance of young leaves on galled plants confirms that almost every node on the 6 test plants was an oviposition site. At these nodes, the gall begins to swell, but any small leaves at that node fail to enlarge further and remain in a stunted state. On the ungalled controls, these small leaves expanded to mature leaves, and small leaves were

![Fig. 4. The appearance of the 6 pairs of Cape-ivy plants at the conclusion of Trial 2. At the start of the Trial, plants were ‘paired’ for similar size and number of leaves. The six ‘treated’ plants (1T, 2T, … 6T) were each exposed for two months to 2 pairs per plant of \( P. \) regalis gall-forming flies. The 6 control plants (1C, 2C, … 6C) were kept in similar cages and not exposed to flies (see Section 2). For each pair, note the smaller size and the fewer leaves of the galled plant on the left, compared to its ‘control’ on the right.](image-url)
found primarily on the new nodes that were produced during the trial.

During Trial 2, the initial size of the Cape-ivy plants was larger (about the size of the larger plants at the end of Trial 1), and each test plant was exposed to far fewer flies—only two pairs each. During this warmer spring period, these larger plants grew much more rapidly, and, unlike Trial 1 when only one control plant developed a new side branch, all 12 plants developed numerous branches. While the stunting of the galled plants was not as dramatic as in Trial 1, the differences were still readily discernible (Fig. 4).

In Trial 2, the cage-within-treatment effect was significant ($P < 0.05$) for some of the response variables, so this term was retained in the ANOVA model for all analyses. These analyses of the results of Trial 2 (Fig. 5) confirmed the same differences as observed in Trial 1. Compared to the ungalled plants, the galled plants: had 48% shorter stems (47.4 dm vs. 90.7 dm, $P = 0.009$) and the stems weighed 40% less (35.1 g vs. 58.8 g, $P = 0.024$), had 35% fewer new nodes (164.5 vs. 251.7, $P = 0.001$), developed 32% fewer large leaves (112.5 vs. 164.3, $P = 0.023$), and the weights of their leaves were 33% less (59.3 g vs. 88.0 g, $P = 0.042$). The mean total weight of the galled plants (114.3 g) was again not statistically different ($P = 0.10$) from the ungalled controls (146.8 g), confirming that biomass production was being diverted to forming galls, chiefly at the expense of producing longer stems and large leaves. Regression analyses of each of these variables against the number of galls, confirmed the statistical differences in the ANOVA analyses.

Although during Trial 2, fly densities were maintained at a level of only 20% of Trial 1, far more galls were formed, a total of 121, with almost all (97%) being mature. The reduced competition for oviposition sites appears to have resulted in more galls reaching maturity. The six exposed plants averaged 20.2 galls per plant ($P = 0.001$), with the galls averaging 19.9 g or 17.5% of the average plant weight.

When gall-forming agents are proposed as biological agents for weeds, it has frequently been speculated that the galls will serve as a “nutrient stress”, or a “metabolic sink”, or “nutrient sink” (Harris, 1980; Maddox, 1982; Harris and Shorthouse, 1996; Turner, 1996). Although nutrient reallocation has been demonstrated for a gall-forming wasp that was successfully used as a weed biocontrol agent (Dennill, 1988), and seed reduction for several Urophora species (Harris, 1980; Story et al., 1989; Piper and Rosenthal, 1995), these results were obtained after the release of the agents. The trials discussed in this paper are one of the few prerelease assessments of potential impact of a prospective, weed-biocontrol agent (see Smith, 2005, for another example), as well as a confirmation of the theory that gall formation modifies plant resource allocation.

These two small trials confirmed that relatively subtle, sublethal impacts on the target can be quantified, even under strict containment conditions. These trials measured the “per-capita effect” of the galls (see McClay and Balciunas, 2005, p. 200), and provided the first estimates for the ‘benefit’ side of a risk analysis when release of these flies is considered. As frequently noted by other authors (e.g., McFadyen, 2003; Sheppard, 2003; McClay and Balciunas, 2005), PREA cannot predict the actual impact an agent will
have after it is released in a novel environment. It remains impossible to predict what population densities this fly might attain in California, if it is released. It might fail to establish, but if it does establish, and _P. regalis_ populations approximate even the lower densities used in Trial 2, then the growth habit of this pest vine should change so that it is less aggressive in clambering over bushes and shrubs. In the field, the actual impact may exceed those observed in our short, laboratory trials. Each of these trials lasted only the life span of the immature stages of a single generation of _P. regalis_. In the field, as many as six generations are possible each year, and the damage from successive generations would accumulate. Likewise, these trials do not measure the impact of the “wounds” left in the plant by the adult flies emerging from the galls. These openings are likely to be utilized by pathogens and other opportunistic natural enemies to cause further damage to Cape-ivy, as well as cause the vines to become more easily water-logged after a rain.

In South Africa, _P. regalis_ are heavily parasitized and the low density of galls makes it difficult to observe their impact on Cape-ivy growth. Although our trials were small and conducted inside a containment greenhouse, they indicate that this fly has good potential to negatively impact Cape-ivy. The results of these two trials encouraged us to complete our host-range tests of _P. regalis_ and then submit a petition for the release of this fly as a biological control agent for Cape-ivy.

Although risk assessments of prospective weed biocontrol agents have historically focused on potential direct effects on nontargets, it is important to also minimize the possibility of indirect nontarget impacts by evaluating the potential efficacy of the candidate agent. As the trials reported here show, a PREA can be performed even under the strict containment conditions of a quarantine facility.

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